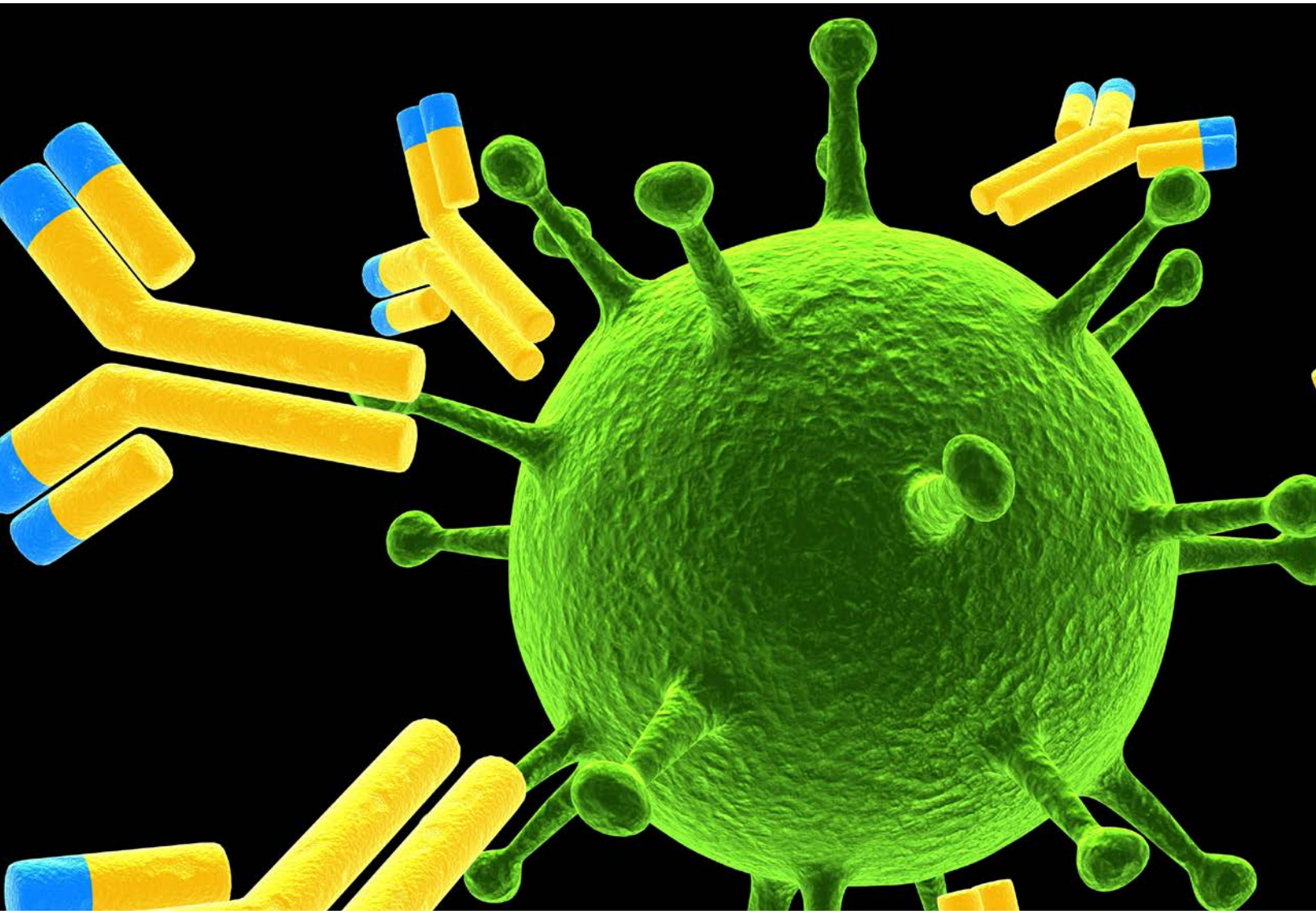
Secondary Detection Probes & Kits



Secondary Antibody Conjugates | Biotin/Streptavidin Conjugates | Enzyme Labeled Conjugates

AAT Bioquest®

Our Mission

AAT Bioquest® is committed to constantly meet or exceed its customer's requirements by providing consistently high quality products and services, and by encouraging continuous improvements in its long-term and daily operations. Our core value is Innovation and Customer Satisfaction.

Our Story

AAT Bioquest®, Inc. develops, manufactures and markets bioanalytical research reagents and kits to life sciences research, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest offers a rapidly expanding list of enabling products. Besides the standard catalog products, we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays and custom high throughput screening of drug discovery targets.

It is my greatest pleasure to welcome you to AAT Bioquest. We greatly appreciate the constant support of our valuable customers. While we continue to rapidly expand, our core value remains the same: Innovation and Customer Satisfaction. We are committed to being the leading provider of novel biological detection solutions. We promise to extend these values to you during the course of our service and to continue to support you with our new products and services. It is our greatest honor to receive valuable feedbacks and suggestions from you so that we can better serve your projects.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

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CUSTOMER SERVICE & ORDERING INFORMATION

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3. Payment: Terms of sale are net 30 days of date of invoice that is sent to you within 24 hours of shipping the order. The amount received must be sufficient to cover both the invoiced amount and any bank charges that may be incurred. Late charges may be added to invoices not paid within the 30-day time period. Late charges must be paid before subsequent orders can be shipped.

4. Warranties: The products shipped by AAT Bioquest are warranted to conform to the chemical or biological descriptions provided in our publications. This warranty is exclusive, and we makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose. Our sole and exclusive liability and your exclusive remedy with respect to products proved to our satisfaction to be defective or nonconforming shall be replacement of such products without charge or refund of the purchase price, in our sole discretion, upon the return of such products in accordance with our instructions. We will not be liable for any incidental, consequential or contingent damages involving their use.

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6. Use of Our Products: Our products are used ONLY for laboratory research and development purposes. We realize that, since our products are, unless otherwise stated, intended primarily for research purposes, they may not be on the Toxic Substances Control Act (TSCA) inventory. You assume responsibility to assure that the products purchased from us are approved for use under TSCA, if applicable. You have the responsibility to verify the hazards and to conduct any further research necessary to learn the hazards involved in using products purchased from us. You also have the duty to warn your customers and any auxiliary personnel (such as freight handlers, etc.) of any risks involved in using or handling the products.

7. Patent Disclaimer: We do not warrant that the use or sale of our products will not infringe the claims of any United States or other patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

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SECONDARY ANTIBODY CONJUGATES

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Introduction

Immunoassays are biochemical tests that measure the presence or concentration of specific proteins, target analytes or other molecules in a biological sample. Immunoassays utilizing secondary detection conjugates or probes come in a variety of different formats and can be applied to an array of techniques such as ELISA, Western Blotting and flow cytometry. Target specific probes are conjugated with a chemical tag (radioactive or enzymatic) or a fluorescent label that generates a detectable and measurable signal (chromogenic or fluorogenic) when in the presence of its target analyte. Immunoassay applications have been widely adopted in many clinical fields as a powerful tool for the diagnosis of disease and therapeutic drug monitoring. The most common types or applications employ a colorimetric or fluorimetric detection system to measure the concentration of the target analyte.

In colorimetric assays, a labeled-reagent in the presence of its target analyte results in a reaction that produces a visible and measurable color change. The intensity of this color change is directly proportional to the concentration of the substance; the more intense the color, the higher the concentration of the analyte. With the aid of a colorimeter, such as a colorimetric microplate reader, the concentration of the particular substance in the solution can be determined by measuring the absorbance of that solution at its maximal absorbance wavelength of light known as lambda max (λmax).

In fluorimetric assays, highly sensitive fluorescently labeled probes are utilized to detect target analytes of a complex sample. A fluorimeter excites the sample of interest at the specific absorption wavelength of the fluorescent dye labeled probe. The excited dye subsequently emits a photon at a greater wavelength as it returns to its ground state. The fluorimeter measures the intensity of the emitted light, which is proportional to the concentration of the analyte. Fluorimetric assays are chosen for their extraordinary sensitivity and high specificity allowing for the precise detection of fluorescent materials in relatively minute or limited sample sizes. They are advantageous for their minimal background interference, brighter signal, and significantly broader dynamic range.

Secondary Detection Probes

Antibodies

Antibodies are the most commonly utilized tool for immunoassay applications. They can be easily conjugated with a fluorescent or chemical label and their high binding affinity for specific antigens enable the detection and isolation of target analytes in a complex biological sample.

Antibodies are large Y-shaped immunoglobulin proteins produced mainly by plasma cells that are recruited by the immune system to neutralize pathogens or foreign objects such as bacteria and viruses. Each antibody has a unique target or protein known as an antigen which is expressed by the invading organism. Foreign antigens elicit an adaptive immune response which stimulates the production of antibodies specifically directed against that pathogen for its detection and removal from the host organism by its immune system.

Key Points

- Antibodies bind to specific antigens and signals to other cells of the immune system to dispose of the invading microbes. Their high affinity for specific targets makes them excellent tools for detection and quantitation assays.
- Immunoglobulins are proteins that function as antibodies and their terms are often used interchangeably. Immunoglobulins are found in blood and other tissues and fluids.
- Paratopes are the antigen-binding sites of antibodies that recognizes and binds to the epitope markers expressed on antigens.

Primary Antibodies

Primary antibodies are immunoglobulins that bind specifically to an antigen. A chemical tag or fluorescent label can be conjugated to a primary antibody for direct detection of a species target antigen.

- **Advantages of Direct Detection:** Quick methodology (only one antibody used) and eliminates the use of non-specific secondary antibodies.
- **Disadvantages of Direct Detection**: Immunoreactivity of the primary antibody may be impeded by its conjugated fluorescent label or chemical tag. Little-to-no signal amplification because typically only one primary antibody will bind to the target analyte in direct detection.

Secondary Antibodies

Secondary antibody conjugates are commonly utilized for indirect detection methods in which a dual antibody detection system is implemented. A complex biological sample is first incubated with a primary antibody that has been directed against a specific antigen or target protein of choice. After removal of excess

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primary antibodies, fluorescently labeled secondary antibodies are employed to detect the presence of the primary antibody, and in doing so indirectly detect the target analyte. Before employment, the sensitivity of the secondary antibody is improved by directing it against the primary antibody's immunoglobulin class or subclass. Upon binding to the primary antibody, the labeled secondary antibody will emit a detectable and measurable fluorescent signal that is an amplification of the primary signal.

Primary antibodies are divided into five major classes, with immunoglobulin G (IgG) being the most abundant. Therefore, a majority of secondary antibodies have been manufactured to target a variety of primary IgG antibodies. Secondary antibodies can be conjugated with a wide variety of labels, including enzymes, biotin, and fluorescent dyes. Species used to generate secondary antibodies should always be different from the primary antibody host and target species. To ensure this, secondary antibodies are generated by immunizing a host animal with an antibody from a different species. For example, an anti-mouse antibody is raised by injecting specific purified mouse antibody into an animal other than a mouse such as a goat.

Secondary antibodies may also be cross-adsorbed to eliminate potential species reactivity. Cross-adsorption is an extra purification step implemented to increase the specificity of a secondary antibody solution by passing the solution of secondary antibodies through a column matrix. This matrix has been immobilized with serum proteins from potential cross reactive species which capture and retain non-

Factors to Consider When Determining the Appropriate Secondary Antibody

Host and Target Species:

- Species used to generate the secondary antibody should always be different from the primary antibody host and target species.

Targeted Reactivity:

- Reactivity includes the target species, the target immunoglobulin (Ig) class/subclass, and whether the secondary antibody binds to a particular part of the antibody.

  ***Note:** Target species are the species from which the primary antibody was derived. This is in contrast to the host species from which the secondary antibody was generated*

Purification:

- Affinity purification processes pass antibody-containing serum through a column matrix containing immobilized affinity ligands which bind the antibody. Intermediate washing steps remove nonessential components of the serum before recovering the "purified" antibody from the column. Affinity purification can be performed using either a ligand that recognizes antibodies (i.e.: Protein G) or a ligand that the antibody detects such as a target antigen.

Cross-Adsorption:

- Increase antibody specificity by eliminating cross-reactivity to other non-target antibodies and proteins. Affinity-purified secondary antibodies are passed through a column containing immobilized antibodies or serum proteins from other species. The secondary antibody which recognize these other species' antibodies or serum proteins will be retained in the column, whereas those without cross-reactivity flow through. Increasing the number of different species of serum components in the adsorption column, will generate antibodies that have cross reactivity to fewer species.

Multiplexing:

- Multiplexing utilizes fluorescent dyes to visualize different targets or components simultaneously. Highly cross-adsorbed antibodies work well in multiplexing because they decrease species cross-reactivity and background interference. *(**Note:** Avoid conjugates with overlapping emission spectra.)*

Conjugates:

- Choice of conjugate depends upon the application and how the secondary antibody is going to be detected. For example, brightness and photostability are parameters to optimize in cell imaging experiments.
  - **iFluor™-Labeled Secondary Antibodies :** exhibit superior brightness and photostability
  - **Classic Fluorescent -Labeled Secondary Antibodies:** FITC, TRITC, Cy3®, Cy5® and Texas Red® dyes conjugated to secondary antibodies. While we strongly recommend using superior iFluor™ dye-conjugates as alternatives, we however do offer traditional conjugates as well. These dyes prove useful is applications which exploit FITC's high rate of photobleaching or pH sensitivity.
  - **Enzyme-Labeled Secondary Antibodies:** enzyme conjugates for secondary detection applications in ELISA and other immunoassays. Enzyme conjugates are used in conjunction with their appropriate chromogenic, fluorogenic or chemiluminescent substrate to create a detectable signal. This is an extremely popular method for analyte quantification.
  - **Biotinylated Secondary Antibodies:** allows for amplification of the target signal, aiding in the detection of a target analyte expressed in low concentrations of complex biological sample. Typically used in conjunction with streptavidin.

Biotin-Binding Proteins:

- For applications where the primary Ab is labeled with a biotin tag (biotinylated IgG), use a biotin-binding protein as a suitable detection reagent.

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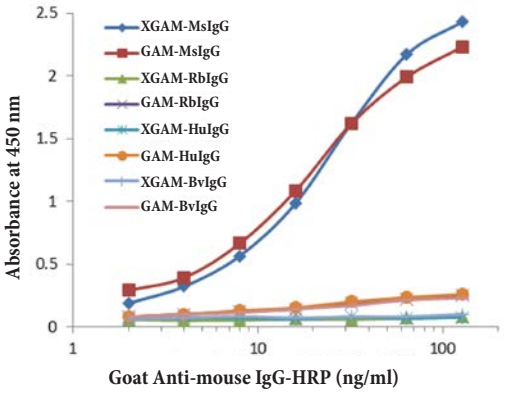

Figure 1.1 Mouse (Ms), rabbit (Rb), human (Hu) and bovine (Bv) IgGs are coated onto an ELISA plate. After washing and blocking, the coated plate is incubated with the affinity purified goat anti-mouse IgG (GAM)-HRP or with pre-absorption goat anti-mouse IgG (XGAM)-HRP at concentrations from 2 to128 ng/ml. The pre-absorbed goat anti-mouse IgG shows the sensitivity in response to mouse IgG at 2 ng/ml with no detectable cross-reaction to other IgGs.

Spectrum Viewer Tool

AAT Bioquest's interactive Spectrum Viewer is a powerful tool helping scientists to easily analyze and compare spectral data. Our Spectrum Viewer includes a comprehensive spectral database consisting of our extensive catalog of fluorophores and fluorophores from other companies. Conveniently providing an instructive one-stop destination for making informed decisions about which products suit their experimental designs and equipment (i.e. lasers and filter sets). Recently, major updates have been made to improve our Spectrum Viewer's overall functionality, added features include:

- A "Share" function to link graph snapshots to other users
- An "Export" function to save graphs as a .PNG file (example of export below Figure 1.2)
- Advanced filtering capabilities to search for compounds by name, excitation/emission ranges and Stokes shift

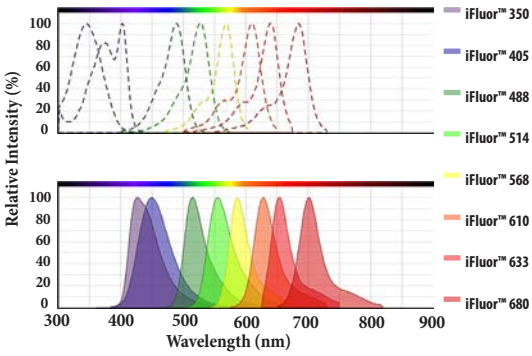

Figure 1.2 Excitation (above) and emission (below) spectra of the iFluor™ dye series.

specific secondary antibodies resulting in highly selective antibodies. These selective secondary antibodies are ideal for multicolor experiments where multiple primary antibodies and their corresponding secondary antibodies are used simultaneously. They may also prove beneficial in immunohistochemistry experiments on samples with abundant amounts of endogenous immunoglobulins (Igs). AAT Bioquest offers a comprehensive line of secondary antibodies conjugated with fluorescent labels and enzymes, as well as biotinylated secondary antibodies. Our secondary antibodies are generated in host species with pooled IgGs from target species. To minimize cross-reaction with other immunoglobulins and improve secondary antibody specificity, we offer a line of affinity purified and cross-adsorbed secondary antibody IgGs (Figure 1.1). These purified secondary antibodies are isolated and separated from pooled antisera of immunized host species via the affinity beads coupled with the IgGs from target species. Our purified IgGs react with all IgG isotypes that are normally present in the total serum of target species.

Features of AAT Bioquest's Secondary Antibodies:

- Reacts with all IgG isotypes of target species with minimal cross-reaction to other, non-target species commonly found in immunoassays.
- Affinity purified with mouse or rabbit IgG coupled beads for high specificity and purity.
- Increased specificity, low background and increased assay sensitivity.
- Cross-Adsorbed for multi-color assays involving the simultaneous usage of several primary antibodies and their respective secondary antibodies.

Conjugation of fluorescent labels to secondary antibodies produces powerful fluorescent probes capable of detecting and measuring the concentration of a specific analyte in a biological sample. These probes posses a wide range of spectra for excitation and emission due to the novel electronic configuration of the fluorescent label conjugated to it. Fluorescently labeled secondary antibodies produce brighter signals, allow for multiplexing capabilities and are simple to use. Applications include multi-color analysis and a wide array of immunoassay techniques such as Western Blotting, flow cytometry and immunohistochemistry.

When Selecting A Fluorescently-Labeled Secondary Antibody For A Particular Application, Consider The Following Factors:

- Select the brightest set of fluorochromes with minimal spectral overlap suitable for your instrument.
- Combine the brightest fluorescent label with the target protein that has the lowest level of expression within a sample and vice versa.
- Secondary antibodies should always be different from the primary antibody host and target species to reduce cross reactions.
- To avoid cross species reactivity in multi-color analysis, consider cross-adsorbed secondary antibodies.
- Consider any potential autofluorescent properties of the biological sample. For example, liver sections autofluoresce in the red channel, therefore avoid staining this tissue type with fluorochromes that emit in the red channel.

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iFluor™ Dyes Conjugated to Secondary Antibodies

AAT Bioquest offers an extensive line of proprietary iFluor™ labeling dyes ideally suited for a broad array of immunoassay applications and for multi-color analysis. Our iFluor™ dyes span the entire spectrum from ultraviolet light to infra-red light while outperforming classic labeling dyes such as the Cy Dyes, Texas Red® and FITC. iFluor™ dyes generate greater fluorescence intensity, have increased photostability and are more water soluble. The fluorescence intensity of iFluor™ labeling dyes remains high over a broad pH range. They experience a minimal quenching effect when coupled to proteins and have been optimized for immunofluorescence and flow cytometry applications. (Table 1 lists the spectral properties of our iFluor™ dyes)

iFluor™ dyes have been conveniently conjugated to secondary antibodies against a diverse set of species. A large number of iFluor™ anti-IgG conjugates have been cross-adsorbed to reduce the risk of any cross-reactivity. Our iFluor™ conjugated secondary antibodies are high quality reagents that are extensively tested to ensure bright staining and low background interference. iFluor™ conjugated secondary antibodies have been tested in a wide range of applications including immunofluorescence, immunocytochemistry, flow cytometry, and fluorescent Western Blot. Data from microscopic and flow cytometric analysis demonstrate that iFluor™-labeled secondary antibody conjugates yield equal or better data quality when compared with the labeled antibodies of other industry leading suppliers (i.e., ThermoFisher's Alexa Fluor® conjugates and Jackson ImmunoResearch Lab's conjugates).

Benefits of iFluor™ Conjugated Secondary Antibodies:

- **Bright Dyes:** the fluorescence intensity of iFluor™ dyes outperform or match other spectrally similar dyes (e.g., Alexa Fluor®)
- **Great Photostability:** Increased photostability allows for longer periods of image capture
- **pH Insensitivity:** iFluor™ fluorescence intensity remains high over a broad pH range
- **Good Water Solubility:** Good water solubility prevents the precipitation and aggregation of iFluor™ conjugated antibodies
- **Optimized & Validated Performance:** Optimized for immunofluorescence microscopy and flow cytometry

Table 1. The complete collection of spectral properties for all iFluor™ secondary antibody conjugates

Fluorophore	Replaces	Excitation (nm)	Emission (nm)
iFluor™ 350	Alexa Fluor® 350, DyLight™ 350, AMCA	345	442
iFluor™ 405	Alexa Fluor® 405, DyLight™ 405	401	420
iFluor™ 488	Alexa Fluor® 488, DyLight™ 488	491	514
iFluor™ 514	Alexa Fluor® 514	518	542
iFluor™ 532	Alexa Fluor® 532	531	556
iFluor™ 546	Alexa Fluor® 546	541	557
iFluor™ 555	Alexa Fluor® 555, DyLight™ 550, Cy3®, TRITC	555	565
iFluor™ 568	Alexa Fluor® 568	568	587
iFluor™ 594	Alexa Fluor® 594, DyLight™ 594, Texas Red®	594	614
iFluor™ 610	Alexa Fluor® 610	605	627
iFluor™ 633	Alexa Fluor® 633, DyLight™ 633	638	655
iFluor™ 647	Alexa Fluor® 647, DyLight™ 650, Cy5®	649	665
iFluor™ 680	Alexa Fluor® 680, DyLight™ 680, Cy5.5®, IRDye® 700	676	695
iFluor™ 700	Alexa Fluor® 700	685	710
iFluor™ 750	Alexa Fluor® 750, DyLight™ 750, Cy7®	749	775
iFluor™ 790	Alexa Fluor® 790, DyLight™ 800, IRDye® 800	782	811
iFluor™ 810	No other commercial equivalents	809	821
iFluor™ 820	No other commercial equivalents	820	825
iFluor™ 830	No other commercial equivalents	832	838
iFluor™ 860	No other commercial equivalents	863	868

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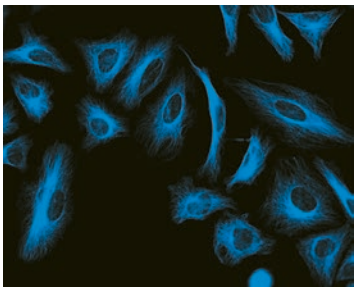

Figure 1.3 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 350 goat anti-mouse IgG (H&L) (Blue, Cat# 16520)

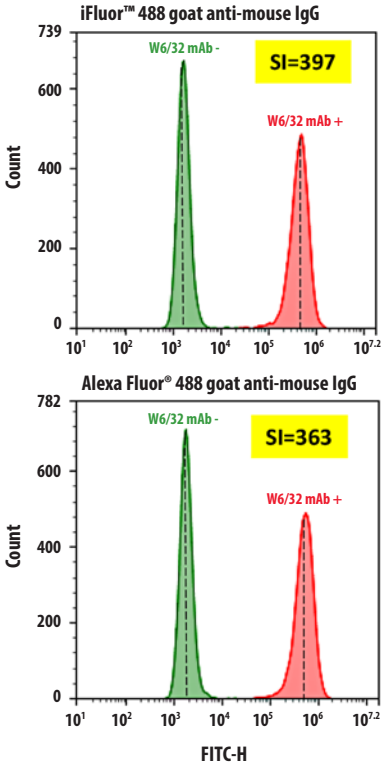

Figure 1.4 HL-60 cells were incubated with (Red, +) or without (Green, -) Anti-human HLA-ABC (W6/32 mAb), followed by iFluor™ 488 goat anti-mouse IgG (Cat# 16528) or Alexa Fluor® 488 goat anti-mouse IgG, respectively. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in FITC channel. The stain index (SI) of each conjugate was calculated. Results indicate that iFluor™ 488 is an excellent replacement for Alexa Fluor® with a slight better signal-to-noise ratio.

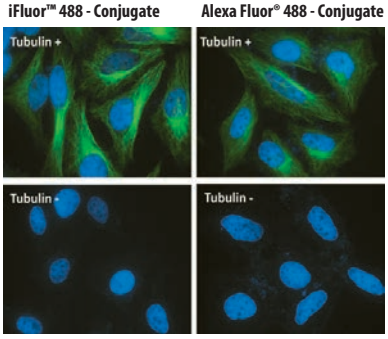

Figure 1.5 Image of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by iFluor™ 488 goat anti-mouse IgG (H&L) (Green, Left, Cat# 16528) or Alexa Fluor® 488 goat anti-mouse IgG (Green, Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

Blue Fluorescent Secondary Antibody Conjugates

iFluor™ 350 Conjugates

A Superior Replacement for AMCA, Alexa Fluor® 350 and DyLight™ 350 Dyes

iFluor™ 350 is a blue-fluorescent dye conjugated to antibodies for secondary detection applications. Its brighter signal intensity and increased photostability makes iFluor™ 350 dye-conjugates a superior and affordable alternative to the poorly water soluble aminomethylcoumarin (AMCA) dye. iFluor™ 350 dyes have spectral properties essentially identical to those of AMCA, DyLight™ 350 and Alexa Fluor® 350 dyes. Its maximum excitation at 345 nm and maximum emission at 442 nm, makes iFluor™ 350 conjugates ideally suited for cytometers equipped with either a 350 nm laser line or DAPI filter. Antibody conjugates prepared with iFluor™ 350 dyes are bright, and their fluorescence is not affected by a pH in the physiological range (pH 4-10). This pH-insensitivity makes iFluor™ 350 dyes favorable for assays requiring extreme pH parameters.

iFluor™ 405 Conjugates

A Superior Replacement for Alexa Fluor® 405 and DyLight™ 405 Dyes

iFluor™ 405 is a blue-fluorescent dye that has an excitation ideally suited to the 405 nm violet laser line found in most new flow cytometers. iFluor™ 405-labeled secondary antibody conjugates have spectral properties nearly identical to those of Alexa Fluor® 405 and BD's Horizon™ BV421 conjugates with a maximum excitation at 401 nm and a maximum emission at 420 nm. These spectral characteristics make iFluor™ 405 conjugates an excellent alternative to the corresponding Alexa Fluor® 405 conjugates. iFluor™ 405 conjugates are bright with moderate photostability and are not significantly affected by pH in the physiological range. For detection and imaging of high-abundance targets like actin or tubulin, iFluor™ 405 conjugates elicit the best results. Compared to other commercially available dye-conjugates in its class, iFluor™ 405 conjugates are one of the brightest fluorescent dye conjugates available in the blue channel. iFluor™ 405 conjugates can be used in conjunction with iFluor™ 488, 594, and 647 dyes for multiplexing applications. In many cases, our iFluor™ 405 secondary antibody conjugates are much brighter than the spectrally similar dye conjugates (e.g., Alexa Fluor® 405 conjugates).

Green Fluorescent Secondary Antibody Conjugates

iFluor™ 488 Conjugates

A Superior Replacement for FITC, DyLight 488™ and Alexa Fluor® 488 Dyes

iFluor™ 488 is a green fluorescent dye that is far superior at labeling proteins than the commonly used fluorescein isothiocyanate (FITC). FITC is pH-sensitive, emitting a maximum fluorescence only at a pH above 9, and is prone to photobleaching during microscopic imaging applications. iFluor™ 488 conjugates have a broader pH range of 4-10, are far more photostable and are significantly brighter than FITC conjugates.

iFluor™ 488 has spectral properties nearly identical to Alexa Fluor® 488. iFluor™ 488

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anti-IgG conjugates have an excitation ideally suited to the 488 nm laser line with a maximum excitation at 491 nm and a maximum emission at 514 nm. Under the same test conditions, iFluor™ 488 secondary antibody conjugates gave equivalent or higher signal-to-noise ratios, making them far superior alternatives than the Alexa Fluor® 488 and FITC-labeled secondary antibody conjugates. Compared to the Alexa Fluor® 488 conjugates that are prepared with the mixed isomers of rhodamine dyes, iFluor™ 488 conjugates are prepared using a highly purified single rhodamine isomer, making the iFluor™ 488 conjugates much more consistent from lot to lot.

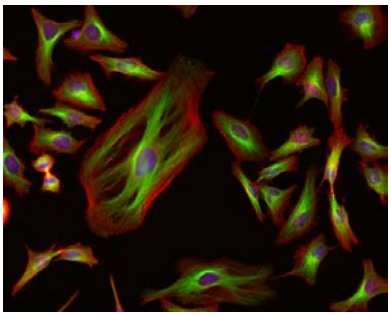

Figure 1.6 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 488 goat anti-mouse IgG (H&L) (Green, Cat# 16528); actin filaments were stained with Phalloidin-iFluor™ 555 Conjugate (Red, Cat# 23119); and nuclei were stained with DAPI (Cat# 17507).

iFluor™ 514 Conjugates

An Excellent Replacement for Alexa Fluor® 514 Dyes

iFluor™ 514 is a stable and bright greenish-yellow fluorescent dye conjugated to antibodies for use in fluorescence imaging and flow cytometry. The iFluor™ 514 anti-IgG fluorescence signal is pH-insensitive from pH 4-10 and exhibits great photostability making it a superior alternative to fluorescein. iFluor™ 514 dye anti-IgG conjugates can be detected with FITC filter sets and have an excitation ideally suited to the 514 nm laser line, making them excellent alternatives to the corresponding Alexa Fluor® 514 and rhodamine 6G-labeled secondary antibody conjugates. iFluor™ 514 has spectral properties almost identical to Alexa Fluor® 514 with a maximum excitation at 518 nm and a maximum emission at 542 nm.

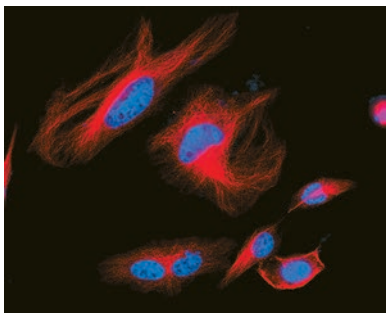

Figure 1.7 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 514 goat anti-mouse IgG (H&L) (Cat# 16532) and nuclei were stained with DAPI (Cat# 17510).

Yellow Fluorescent Secondary Antibody Conjugates

iFluor™ 532 Conjugates

An Excellent Replacement for Alexa Fluor® 532 Dyes

iFluor™ 532 is a yellow-fluorescent dye with an excitation ideally suited for the frequency-doubled Nd:YAG laser line. iFluor™ 532 dye is pH-insensitive over a wide range, ensuring a stable signal in live cell imaging and flow cytometry applications. It is particularly useful in multicolor applications, including super-resolution microscopy using dSTORM. iFluor™ 532 anti-IgG conjugates have an excitation ideally suited to the 532 nm laser line, making them excellent alternatives to the corresponding Alexa Fluor® 532-labeled secondary antibody conjugates. iFluor™ 532 has spectral properties nearly identical to Alexa Fluor® 532 with a maximum excitation at 542 nm and a maximum emission at 558 nm.

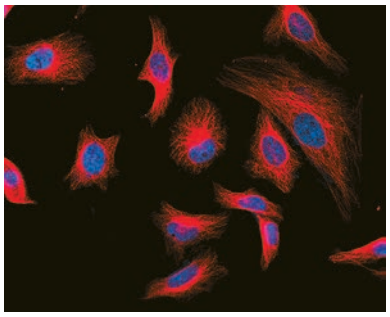

Figure 1.8 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 532 goat anti-mouse IgG (H&L) (Red, Cat# 16536) and nuclei were stained with DAPI (Blue, Cat# 17510).

Orange Fluorescent Secondary Antibody Conjugates

iFluor™ 546 Conjugates

A Superior Replacement for Alexa Fluor® 546 Dyes

iFluor™ 546-labeled anti-IgG conjugates exhibit a bright orange fluorescence signal and great photostability. Used for its stable signal generation in imaging and flow cytometry, the fluorescence intensity of iFluor™ 546 conjugates is pH-insensitive from a range of pH 4-11. The iFluor™ 546-labled antibody conjugates can be well excited with either Nd:YAG laser (~532 nm) or Helium-Neon laser (~543 nm). iFluor™ 546 family has spectral properties essentially identical to those of Alexa Fluor® 546. Under

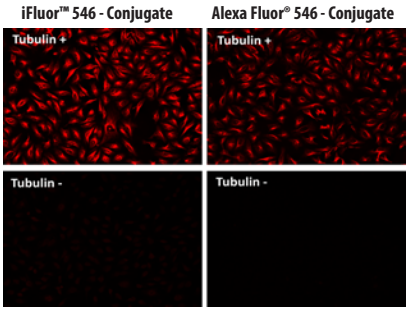

Figure 1.9 Image of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by iFluor™ 546 goat anti-mouse IgG (H&L) (Left, Red, Cat# 16536) or Alexa Fluor® 546 goat anti-mouse IgG (Red, Right), respectively.

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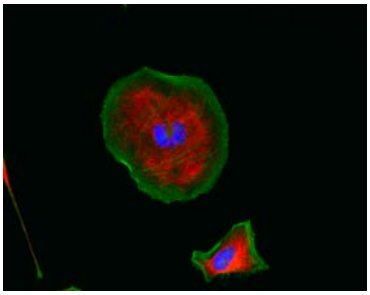

Figure 1.10 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 555 goat anti-mouse IgG (H&L) (Red, Cat# 16540); actin filaments were stained with Phalloidin-iFluor™ 488 Conjugate (Green, Cat# 23115); and nuclei were stained with DAPI (Blue, Cat# 17507).

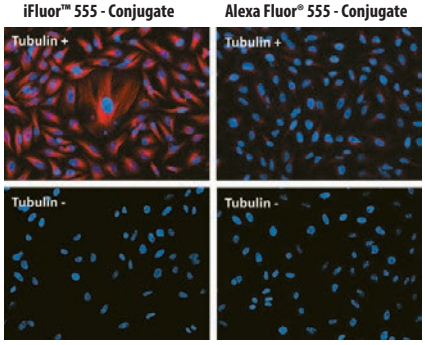

Figure 1.11 Image of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by iFluor™ 555 goat anti-mouse IgG (H&L) (Red, Left, Cat# 16540) or Alexa Fluor® goat anti-mouse IgG (Red, Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

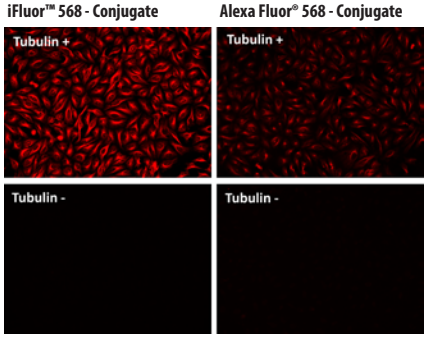

Figure 1.12 Image of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by iFluor™ 568 goat anti-mouse IgG (H&L) (Left, Red, Cat# 16536) or Alexa Fluor® 568 goat anti-mouse IgG (Red, Right), respectively.

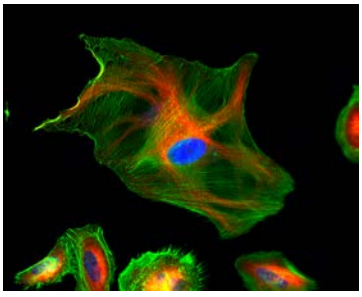

Figure 1.13 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 594 goat anti-mouse IgG (H&L) (Red: Cat# 16548); actin filaments were stained with Phalloidin-iFluor™ 488 Conjugate (Green: Cat# 23115); and nuclei were stained with DAPI (Cat# 17507).

identical test conditions, iFluor™ 546 antibody conjugates are brighter and more photostable than the corresponding Alexa Fluor® 546 conjugates. In addition, iFluor™ 546 secondary antibody conjugates gave a higher signal-to-background ratio than the corresponding Alexa Fluor® 546-labeled conjugates. These spectral and labeling characteristics make the iFluor™ 546 dye family a superior alternative to Alexa Fluor® 546.

iFluor™ 555 Conjugates

A Superior Replacement for Cy3®, DyLight™ 550 and Alexa Fluor® 555 Dyes

iFluor™ 555 is a bright orange fluorescent dye which demonstrates great photostability. Used for their stable signal generation in imaging and flow cytometry, the fluorescence intensity of iFluor™ 555 conjugates are pH-insensitive from pH 4-11. The iFluor™ 555-labeled anti-IgG conjugates can be well excited with either the Nd:YAG laser (~532 nm), Helium-Neon laser (~543 nm) or Krypton ion laser (~568 nm). The iFluor™ 555 family has spectral properties essentially identical to those of Cy3® and Alexa Fluor® 555 with a maximum excitation at 559 nm and a maximum emission at 569 nm. Under identical test conditions, iFluor™ 555 secondary antibody conjugates gave a higher signal-to-noise ratio than the corresponding Alexa Fluor® 555-labeled conjugates. In addition, iFluor™ 555-labeled conjugates are much more photostable than the corresponding Cy3® and Alexa Fluor® 555-labeled conjugates. These spectral characteristics make the iFluor™ 555 family a superior alternative to Cy3® and Alexa Fluor® 555.

Red Fluorescent Secondary Antibody Conjugates

iFluor™ 568 Conjugates

A Superior Replacement for Texas Red®, DyLight™ 594 and Alexa Fluor® 568 Dyes

iFluor™ 568-labeled anti-IgG conjugates exhibit a bright red fluorescence signal and great photostability. Used for their stable signal generation in imaging and flow cytometry, the fluorescence intensity of iFluor™ 568 conjugates is pH-insensitive from a range of pH 4-11. The iFluor™ 568-labeled antibody conjugates can be well excited with the Krypton ion laser (~568 nm). The iFluor™ 568 family has spectral properties essentially identical to those of Alexa Fluor® 568. Under identical test conditions, iFluor™ 568 antibody conjugates were brighter and more photostable than the corresponding Alexa Fluor® 568 conjugates. In addition, iFluor™ 568 secondary antibody conjugates gave a higher signal-to-background ratio than the corresponding Alexa Fluor® 568-labeled conjugates. These spectral and labeling characteristics make the iFluor™ 568 dye family a superior alternative to Alexa Fluor® 568.

iFluor™ 594 Conjugates

A Superior Replacement for Texas Red®, DyLight™ 594 and Alexa Fluor® 594 Dyes

iFluor™ 594 has spectral characteristics similar to those of Texas Red®, DyLight™ 594 and Alexa Fluor® 594 with a maximum excitation at 592 nm and a maximum emission at 614 nm when conjugated to proteins such as antibodies. iFluor™ 594 dyes have superior labeling performance and better stability than Texas Red®. Our

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iFluor™ 594 secondary antibody conjugates provide a high fluorescence intensity and a low background interference as validated in the immunofluorescence staining of mammalian cells. iFluor™ 594 conjugates exhibit little spectral overlap with green-fluorescent conjugates, and can be effectively excited by the 568 nm laser line of the Ar-Kr laser and by the 594 nm laser line of the orange He-Ne lasers. This minimal spectral overlap makes iFluor™ 594 an ideal second color in combination with a green color such as GFP, FITC, or iFluor™ 488.

iFluor™ 633 Conjugates

A Superior Replacement for DyLight™ 633 and Alexa Fluor® 633 Dyes

iFluor™ 633 secondary antibody conjugates are spectrally similar to Alexa Fluor® 633 and DyLight™ 633 conjugates. Fluorescence emission of iFluor™ 633 dyes are well separated from that of other commonly used red fluorophores, such as TAMRA, Texas Red®, Alexa Fluor® 594, iFluor™ 594 and R-phycoerythrin. iFluor™ 633 dyes have an excitation ideally suited to the 633 nm red laser in flow cytometers with a maximum excitation at 638 nm and a maximum emission at 655 nm, giving a deep red emission. Compared to Alexa Fluor® 633, the extinction coefficient of iFluor™ 633 is much higher (~250,000 $cm^{-1}M^{-1}$).

iFluor™ 647 Conjugates

A Superior Replacement for Cy5®, DyLight™ 650 and Alexa Fluor® 647 Dyes

iFluor™ 647-labeled anti-IgG conjugates exhibit a bright red fluorescence signal, great photostability, and minimal quenching on proteins. Used for their stable signal generation in imaging and flow cytometry, the fluorescence intensity of iFluor™ 647 conjugates are pH-insensitive from a range of pH 4-11. The iFluor™ 647-labeled anti-IgG conjugates can be well excited with either a Helium-Neon laser (~633 nm), a red diode laser (~635 nm) or a Krypton ion laser (~647 nm). iFluor™ 647 conjugates have spectral properties essentially identical to those of Cy5® and Alexa Fluor® 647. Under identical test conditions, iFluor™ 647 secondary antibody conjugates are brighter, and gave a higher signal-to-background ratio than the corresponding Cy5® and Alexa Fluor® 647-labeled conjugates. These spectral characteristics make this iFluor™ dye family a superior alternative to Cy5® and Alexa Fluor® 647.

iFluor™ 680 Conjugates

A Superior Replacement for Cy5.5®, IRDye® 700 and Alexa Fluor® 680 Dyes

iFluor™ 680 is a bright, near-IR fluorescent dye with an excitation ideally suited for either a Helium-Neon laser (~633 nm), a red diode laser (~635 nm) or a Krypton ion laser (~647 nm). Used for their stable signal generation in imaging and flow cytometry, iFluor™ 680 conjugates are pH-insensitive over a range from pH 4-11. The long wavelength emission of iFluor™ 680 anti-IgG conjugates are advantageous for detecting target analytes in complex samples with competing auto-fluorescent background signals. iFluor™ 680-labled anti-IgG conjugates exhibit bright fluorescent signals, great photostability and have minimal quenching when conjugated to proteins. The iFluor™ 680 family has spectral properties essentially identical to those

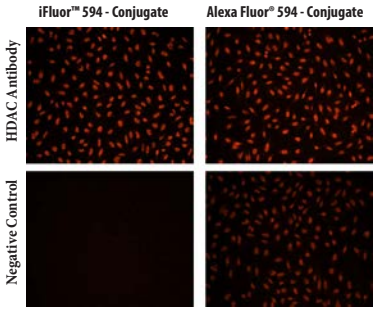

Figure 1.14 Image comparison of HeLa cells. HeLa cells were stained with Rabbit HDAC antibody, and then followed with iFluor™ 594 Goat Anti-Rabbit IgG (H&L) (Left, Cat# 16698) and Alexa Fluor® 594 goat anti-rabbit IgG respectively under the same conditions. The iFluor™ 594 goat anti-rabbit IgG conjugate (left panel) demonstrated much lower staining background than the corresponding Alexa Fluor® 594 (right panel).

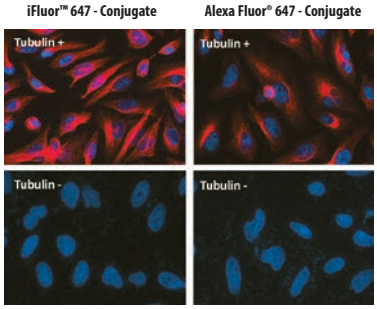

Figure 1.15 Image of HeLa cells. HeLa cells were incubated with (Tubulin+) or without (Tubulin-) mouse anti-tubulin then followed with iFluor™ 647 goat anti-mouse IgG (H&L) (Left, Cat# 16562) or Alexa Fluor® goat anti-mouse IgG (Red), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17530).

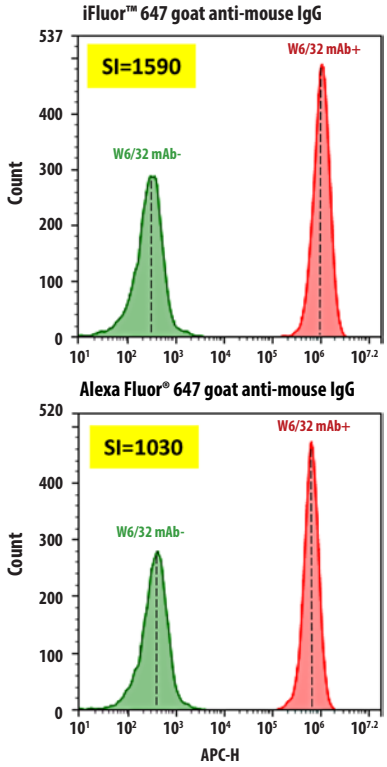

Figure 1.16 HL-60 cells were incubated with (Red, +) or without (Green, -) Anti-human HLA-ABC (W6/32 mAb), followed by iFluor™ 647 goat anti-mouse IgG (H&L) (Cat# 16562) or Alexa Fluor® 647 goat anti-mouse IgG conjugate (Green), respectively. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the APC channel. The stain index (SI) of each conjugate was calculated. Because of iFluor™ 647 conjugates enhanced signal-to-noise ratio, indicative of its larger SI, it is a more sensitive and superior replacement for Alexa Fluor® 647 conjugates.

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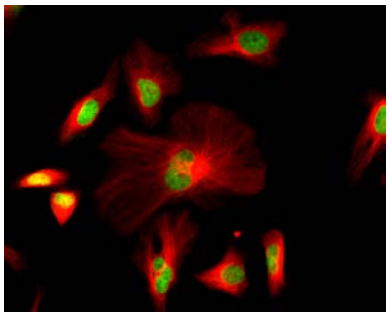

Figure 1.17 Image of HeLa cells. HeLa cells were stained with iFluor™ 680 goat anti-mouse IgG (H&L) (Red, Cat# 16566), and nuclei were stained with Nuclear Green™ DCS1 (Green, Cat# 17550).

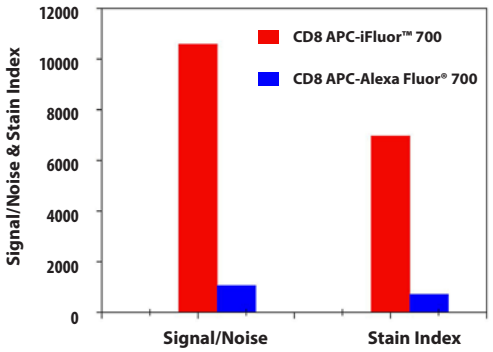

Figure 1.18 Flow cytometric analysis of APC-iFluor ™700 (Red Bar, Cat# 2570) or APC-Alexa Fluor® 700 (Blue Bar) anti-human CD8 on human lymphocytes. Whole blood was stained with APC-iFluor ™700 or APC-Alexa Fluor® 700 anti-human CD8 and compared to whole blood stained with a APC-iFluor ™700 and APC-Alexa Fluor® 700 mouse IgG control. Flow cytometry was performed on a ACEA flow cytometry system.

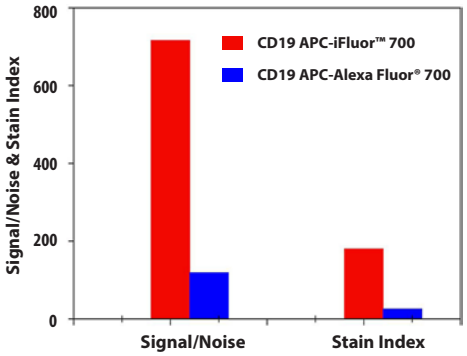

Figure 1.19 Flow cytometric analysis of APC-iFluor ™700 (Red Bar, Cat# 2570) or APC-Alexa Fluor® 700 (Blue Bar) anti-human CD19 on human lymphocytes. Whole blood was stained with APC-iFluor ™700 or APC-Alexa Fluor® 700 anti-human CD19 and compared to whole blood stained with a APC-iFluor ™700 and APC-Alexa Fluor® 700 mouse IgG control. Flow cytometry was performed on a ACEA flow cytometry system.

of Cy5.5® and Alexa Fluor® 680. These spectral characteristics make this new dye family a superior alternative to Cy5.5® and Alexa Fluor® 680.

iFluor™ 700 Conjugates

A Superior Replacement for Alexa Fluor® 700 Dyes

iFluor™ 700 is a far-red dye that can be excited with 633–650 nm lasers. iFluor™ 700 conjugates have excitations ideally suited for either a Helium-Neon laser (~633 nm), a red diode laser (~635 nm) or a Krypton ion laser (~647 nm). This enables multicolor analysis in conjunction with antibodies labeled with APC or iFluor™ 647, and APC-Cy7® reagents. Used for its signal generation in imaging and flow cytometry, iFluor™ 700-labeled secondary antibody conjugates are pH-insensitive over a wide range from pH 4-11. The long wavelength emission of iFluor™ 700 anti-IgG conjugates are favorable for detecting target analytes in complex samples with competing auto-fluorescent background signals. iFluor™ 700-labeled anti-IgG conjugates exhibit bright fluorescent signals and great photostability. The iFluor™ 700 family has spectral properties nearly identical to those of Alexa Fluor® 700. When compared to Alexa Fluor® 700-labeled antibodies, the spectral characteristics and significantly higher signal-to-background ratio of iFluor™ 700-labled antibodies, makes iFluor™ 700 conjugates a far superior alternative to Alexa Fluor® 700 conjugates.

Fluorescence Instruments

There are four primary types of instruments that measure fluorescence: spectrofluorometers such as microplate readers, flow cytometers, fluorescence scanners and fluorescence microscopes. All of which require the following key elements:

Excitation Source

- UV-visible lamps (e.g. LED) or lasers are used to provide the energy required to excite specific fluorophores.

Light Collection Optics

- Researches can utilize any ideal combination of optical elements such as lenses, mirrors and filters to develop an efficient imaging system suitable for their experimental needs.

Detection, Amplification and Digitization

- Photomultiplier tubes (PMT) or a charged-coupled device (CCD) are used for the detection and quantification of emitted light by converting emitted fluorescent light into measurable electrical energy.

Product Ordering Information For iFlour™-Secondary Antibody Conjugates

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
16440	iFluor™ 350 goat anti-mouse IgG (H+L)	200 µg	345	442
16730	iFluor™ 350 goat anti-mouse IgG (H+L)	1 mg	345	442
16520	iFluor™ 350 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	345	442
16770	iFluor™ 350 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	345	442
16600	iFluor™ 350 goat anti-rabbit IgG (H+L)	200 µg	345	442

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Product Ordering Information For iFluor™-Secondary Antibody Conjugates

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
16795	iFluor™ 350 goat anti-rabbit IgG (H+L)	1 mg	345	442
16670	iFluor™ 350 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	345	442
16825	iFluor™ 350 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	345	442
16444	iFluor™ 405 goat anti-mouse IgG (H+L)	200 µg	401	420
16731	iFluor™ 405 goat anti-mouse IgG (H+L)	1 mg	401	420
16524	iFluor™ 405 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	401	420
16771	iFluor™ 405 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	401	420
16604	iFluor™ 405 goat anti-rabbit IgG (H+L)	200 µg	401	420
16796	iFluor™ 405 goat anti-rabbit IgG (H+L)	1 mg	401	420
16674	iFluor™ 405 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	401	420
16826	iFluor™ 405 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	401	420
16448	iFluor™ 488 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	491	514
16735	iFluor™ 488 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	491	514
16528	iFluor™ 488 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	491	514
16773	iFluor™ 488 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	491	514
16608	iFluor™ 488 goat anti-rabbit IgG (H+L)	200 µg	491	514
16800	iFluor™ 488 goat anti-rabbit IgG (H+L)	1 mg	491	514
16678	iFluor™ 488 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	491	514
16828	iFluor™ 488 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	491	514
16452	iFluor™ 514 goat anti-mouse IgG (H+L)	200 µg	518	542
16736	iFluor™ 514 goat anti-mouse IgG (H+L)	1 mg	518	542
16532	iFluor™ 514 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	518	542
16774	iFluor™ 514 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	518	542
16612	iFluor™ 514 goat anti-rabbit IgG (H+L)	200 µg	518	542
16801	iFluor™ 514 goat anti-rabbit IgG (H+L)	1 mg	518	542
16682	iFluor™ 514 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	518	542
16829	iFluor™ 514 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	518	542
16456	iFluor™ 532 goat anti-mouse IgG (H+L)	200 µg	531	556
16737	iFluor™ 532 goat anti-mouse IgG (H+L)	1 mg	531	556
16536	iFluor™ 532 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	531	556
16775	iFluor™ 532 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	531	556
16616	iFluor™ 532 goat anti-rabbit IgG (H+L)	200 µg	531	556
16802	iFluor™ 532 goat anti-rabbit IgG (H+L)	1 mg	531	556
16686	iFluor™ 532 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	531	556
16830	iFluor™ 532 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	531	556
16457	iFluor™ 546 goat anti-mouse IgG (H+L)	200 µg	541	557
16537	iFluor™ 546 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	541	557
16618	iFluor™ 546 goat anti-rabbit IgG (H+L)	200 µg	541	557
16688	iFluor™ 546 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	541	557
16460	iFluor™ 555 goat anti-mouse IgG (H+L)	200 µg	559	569
16739	iFluor™ 555 goat anti-mouse IgG (H+L)	1 mg	559	569
16540	iFluor™ 555 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	559	569
16776	iFluor™ 555 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	559	569
16620	iFluor™ 555 goat anti-rabbit IgG (H+L)	200 µg	559	569
16803	iFluor™ 555 goat anti-rabbit IgG (H+L)	1 mg	559	569
16690	iFluor™ 555 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	559	569

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Product Ordering Information For iFlour™-Secondary Antibody Conjugates

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
16831	iFluor™ 555 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	559	569
16462	iFluor™ 568 goat anti-mouse IgG (H+L)	200 µg	568	587
16541	iFluor™ 568 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	568	587
16622	iFluor™ 568 goat anti-rabbit IgG (H+L)	200 µg	568	587
16692	iFluor™ 568 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	568	587
16468	iFluor™ 594 goat anti-mouse IgG (H+L)	200 µg	592	614
16741	iFluor™ 594 goat anti-mouse IgG (H+L)	1 mg	592	614
16548	iFluor™ 594 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	592	614
16780	iFluor™ 594 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	592	614
16628	iFluor™ 594 goat anti-rabbit IgG (H+L)	200 µg	592	614
16806	iFluor™ 594 goat anti-rabbit IgG (H+L)	1 mg	592	614
16698	iFluor™ 594 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	592	614
16833	iFluor™ 594 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	592	614
16478	iFluor™ 633 goat anti-mouse IgG (H+L)	200 µg	638	655
16743	iFluor™ 633 goat anti-mouse IgG (H+L)	1 mg	638	655
16558	iFluor™ 633 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	638	655
16782	iFluor™ 633 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	638	655
16638	iFluor™ 633 goat anti-rabbit IgG (H+L)	200 µg	638	655
16808	iFluor™ 633 goat anti-rabbit IgG (H+L)	1 mg	638	655
16704	iFluor™ 633 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	638	655
16835	iFluor™ 633 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	638	655
16482	iFluor™ 647 goat anti-mouse IgG (H+L)	200 µg	654	674
16744	iFluor™ 647 goat anti-mouse IgG (H+L)	1 mg	654	674
16562	iFluor™ 647 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	654	674
16783	iFluor™ 647 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	654	674
16642	iFluor™ 647 goat anti-rabbit IgG (H+L)	200 µg	654	674
16809	iFluor™ 647 goat anti-rabbit IgG (H+L)	1 mg	654	674
16710	iFluor™ 647 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	654	674
16837	iFluor™ 647 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	654	674
16486	iFluor™ 680 goat anti-mouse IgG (H+L)	200 µg	682	701
16745	iFluor™ 680 goat anti-mouse IgG (H+L)	1 mg	682	701
16566	iFluor™ 680 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	682	701
16784	iFluor™ 680 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	682	701
16646	iFluor™ 680 goat anti-rabbit IgG (H+L)	200 µg	682	701
16810	iFluor™ 680 goat anti-rabbit IgG (H+L)	1 mg	682	701
16712	iFluor™ 680 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	682	701
16838	iFluor™ 680 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	682	701
16494	iFluor™ 700 goat anti-mouse IgG (H+L)	200 µg	693	713
16746	iFluor™ 700 goat anti-mouse IgG (H+L)	1 mg	693	713
16574	iFluor™ 700 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	693	713
16785	iFluor™ 700 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	693	713
16652	iFluor™ 700 goat anti-rabbit IgG (H+L)	200 µg	693	713
16811	iFluor™ 700 goat anti-rabbit IgG (H+L)	1 mg	693	713
16714	iFluor™ 700 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	693	713
16839	iFluor™ 700 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	693	713

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Near Infrared and Infrared Secondary Antibody Conjugates

Chemiluminescence is a widely popular western blot detection method due to its high sensitivity and versatile detectability with film or digital imaging equipment such as a cooled charge coupled device (CCD) camera. This detection method utilizes enzyme-conjugated secondary antibodies that produce a detectable luminescent signal when reacted with its appropriate substrate. Chemiluminescent signals are short-lived and are detectable only for the duration of the enzyme-substrate reaction. Once the substrate has been completely depleted or the reporter enzyme loses its activity, then the reaction will cease and the signal will be terminated. Traditional methods used to capture these transient chemiluminescent signals are x-ray films. Unfortunately, the continuously increasing cost of x-ray components for chemiluminescence detection has led to the transition and development of more sensitive and technologically advanced digital imaging systems and CCD cameras.

CCD cameras are versatile in their ability to capture both chemiluminescent and fluorescent images. Howbeit, there are limitations associated with both methods of imaging. Chemiluminescence detected by a CCD camera records the photons and displays an image based on the amount of light generated as a result of a dynamic chemical reaction. Dynamic enzymatic reactions are continuously changing overtime making it crucial to optimize reaction times and imaging when performing chemiluminescence detection with a CCD camera. Fluorescence detection with a CCD camera alleviates this problem because the signal produced is measured at a static state making for a more precise and accurate measurement. Unfortunately, this method of detection exhibits insufficient performance from fluorophores in the visible light spectrum. This poor performance is attributed to the high background interference (light scattering and autofluorescence) produced by the biological components and membranes used in fluorescent imaging systems of the visible wavelength range. The need for a solution to these complications in chemiluminescent and fluorescent detection of the UV-spectrum has sparked the development of near infrared and infrared imaging systems.

Near infrared and infrared imaging systems measure the signal generated in a static state eliminating precautionary steps needed to optimize detection of dynamic chemiluminescent reactions. Fluorophores detected at the near infrared and infrared wavelengths provide higher sensitivity than those detected in the visible light spectrum. Autofluorescence from membrane surfaces and biomolecules is significantly reduced at longer wavelengths, providing decreased background interference and improving sensitivity. In addition, near infrared and infrared imaging systems have a wider linear detection range, increased stability, and can be optimized for multiplexing. A wide linear detection range in combination with the static state of fluorescence detection allows for all protein concentrations of the sample within the instrument's detectable range to be made visible.

AAT Bioquest offers one of the largest collection of near infrared and infrared fluorescent dyes conjugated to secondary antibodies for use in applications such as Western Blotting and in vivo imaging. AAT Bioquest is the only major commercial

Chemiluminescence vs. Infrared Fluorescence

Multiplexing:

- **Chemiluminescence:** **No**
- **Infrared Fluorescence:** **Yes**

Advantage: Infrared western blot allows normalization or comparative analysis without stripping and reprobing of blots.

Detection:

- **Chemiluminescence:** **Indirect**

Disadvantage: in chemiluminescence enzyme/substrate kinetics may affect performance.

- **Infrared Fluorescence:** **Direct**

Advantage: using infrared iFluor™-labeled antibodies signal is directly proportional to the amount of target protein

Stability:

- **Chemiluminescence:** **Hours**
- **Infrared Fluorescence:** **Months To Years**

Advantage: iFluor™ dye fluorescent signal is highly stable, so you can store blots and re-image later.

Sensitivity:

- **Chemiluminescence:** **-**
- **Infrared Fluorescence:** **+**

Advantage: infrared detection is static allowing for a wider linear detection range, compared to chemiluminescence, without any signal loss. Additionally, IR imaging can be performed simultaneously on the same blot for improved detection efficiency.

Time:

- **Chemiluminescence:** **More**
- **Infrared Fluorescence:** **Less**

Advantage: infrared iFluor™ secondary antibodies have wide dynamic range to save your time by reducing the need of multiple exposures.

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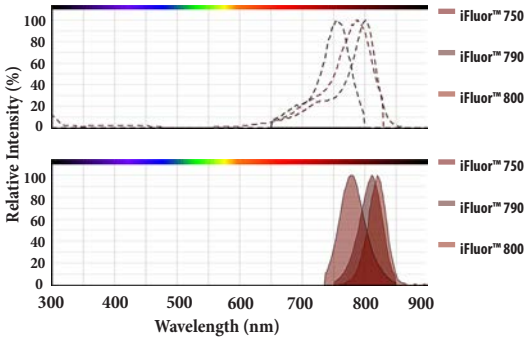

Figure 1.20 Excitation (above) and emission (below) spectra of the near infrared and infrared iFluor™ 750, 790 and 800 dye series.

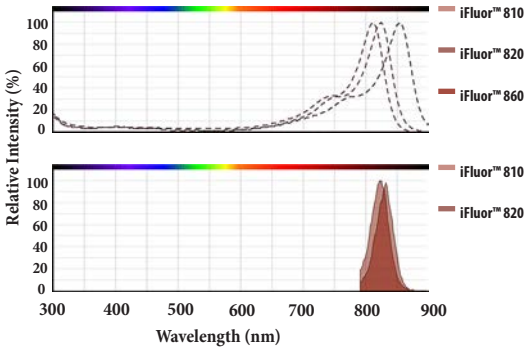

Figure 1.21 Excitation (above) and emission (below) spectra of the infrared iFluor™ 810, 820 and 860 dye series.

source for fluorescent dyes exhibiting a maximum absorption wavelength longer than 820 nm. Our iFluor™ 750 and 790 secondary antibody conjugates are optimized for near infrared and infrared Western Blotting application.

Product Ordering Information For AAT Bioquest's Newest Infrared Fluorescent Labeling Probes.

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
1375	iFluor™ 800 acid	1 mg	809	821
1378	iFluor™ 800 maleimide	1 mg	809	821
1379	iFluor™ 800 succinimidyl ester	1 mg	809	821
1385	iFluor™ 810 acid	1 mg	820	825
1388	iFluor™ 810 maleimide	1 mg	820	825
1389	iFluor™ 810 succinimidyl ester	1 mg	820	825
1395	iFluor™ 820 acid	1 mg	832	838
1398	iFluor™ 820 maleimide	1 mg	832	838
1399	iFluor™ 820 succinimidyl ester	1 mg	832	838
1405	iFluor™ 860 acid	1 mg	868	868
1408	iFluor™ 860 maleimide	1 mg	868	868
1409	iFluor™ 860 succinimidyl ester	1 mg	868	868

Product Ordering Information For Infrared iFlour™-Secondary Antibody Conjugates

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
16506	iFluor™ 750 goat anti-mouse IgG (H+L)	200 µg	753	779
16748	iFluor™ 750 goat anti-mouse IgG (H+L)	1 mg	753	779
16586	iFluor™ 750 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	753	779
16788	iFluor™ 750 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	753	779
16660	iFluor™ 750 goat anti-rabbit IgG (H+L)	200 µg	753	779
16813	iFluor™ 750 goat anti-rabbit IgG (H+L)	1 mg	753	779
16720	iFluor™ 750 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	753	779
16842	iFluor™ 750 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	753	779
16507	iFluor™ 790 goat anti-mouse IgG (H+L)	200 µg	782	811
16750	iFluor™ 790 goat anti-mouse IgG (H+L)	1 mg	782	811
16587	iFluor™ 790 goat anti-mouse IgG (H+L) *Cross Adsorbed*	200 µg	782	811
16790	iFluor™ 790 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	782	811
16661	iFluor™ 790 goat anti-rabbit IgG (H+L)	200 µg	782	811
16815	iFluor™ 790 goat anti-rabbit IgG (H+L)	1 mg	782	811
16721	iFluor™ 790 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	200 µg	782	811
16843	iFluor™ 790 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	782	811

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trFluor™ Secondary Antibody Conjugates

Many biological compounds present in cells, serum or other biological samples are naturally fluorescent. Therefore, the use of conventional fluorophores leads to serious limitations in assay sensitivity due to the high background interference caused by the autofluorescence of the biological molecules being assayed. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes short-lived fluorescent interferences.

Time-Resolved fluorescence spectroscopy provides a deeper insight in regards to the molecular environment of a fluorophore through the exploitation of the time-dependent nature of fluorescence. AAT Bioquest's trFluor™ probes enable time-resolved fluorimetry for assays requiring high sensitivity. trFluor™ secondary antibody conjugates possess large Stokes shifts and extremely long emission half-lives when compared with other commercially available fluorophore conjugates. Furthermore, trFluor™ probes exhibit relatively high stability, high emission yield and the ability to be conjugated to biomolecules.

AAT Bioquest offers trFluor™-labeled secondary antibody conjugates for developing time-resolved fluorescence based assays. trFluor™ dyes are covalently linked to anti-mouse IgG and anti-rabbit IgG for their intended use as second step reagents for indirect immunofluorescent staining. trFluor™-labeled secondary antibodies are used in conjunction with primary antibodies.

trFluor™ Dyes For TR-FRET Assays

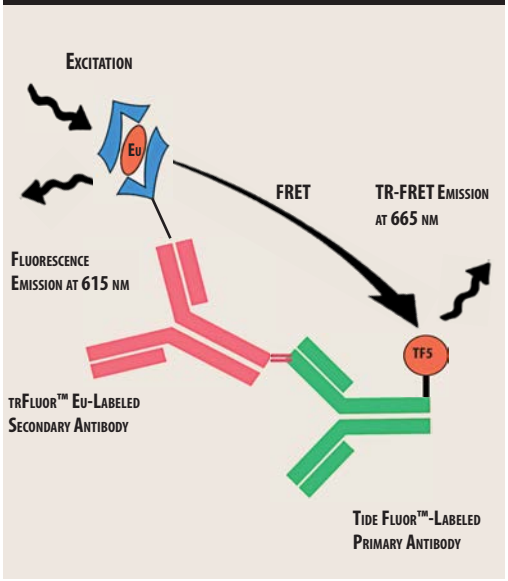

Figure 1.22 TR-FRET Assay Principle using trFluor™ Eu as the donor while Tide Fluor™ 5 (TF5) as the acceptor

Product Ordering Information For trFluor™-Secondary Antibody Conjugates

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
16518	trFluor™ Eu goat anti-mouse IgG (H+L)	100 µg	346	617
16755	trFluor™ Eu goat anti-mouse IgG (H+L)	1 mg	346	617
16598	trFluor™ Eu goat anti-mouse IgG (H+L) *Cross Adsorbed*	100 µg	346	617
16791	trFluor™ Eu goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	346	617
16668	trFluor™ Eu goat anti-rabbit IgG (H+L)	100 µg	346	617
16820	trFluor™ Eu goat anti-rabbit IgG (H+L)	1 mg	346	617
16725	trFluor™ Eu goat anti-rabbit IgG (H+L) *Cross Adsorbed*	100 µg	346	617
16847	trFluor™ Eu goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	346	617
16519	trFluor™ Tb goat anti-mouse IgG (H+L)	100 µg	330	544
16756	trFluor™ Tb goat anti-mouse IgG (H+L)	1 mg	330	544
16599	trFluor™ Tb goat anti-mouse IgG (H+L) *Cross Adsorbed*	100 µg	330	544
16792	trFluor™ Tb goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	330	544
16669	trFluor™ Tb goat anti-rabbit IgG (H+L)	100 µg	330	544
16821	trFluor™ Tb goat anti-rabbit IgG (H+L)	1 mg	330	544
16726	trFluor™ Tb goat anti-rabbit IgG (H+L) *Cross Adsorbed*	100 µg	330	544
16848	trFluor™ Tb goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	330	544

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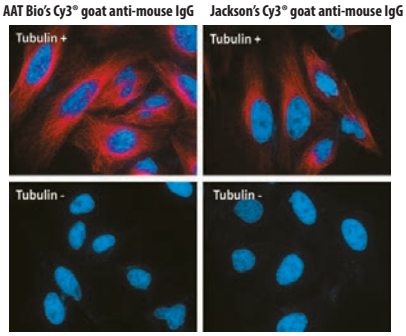

Figure 1.23 Image comparison of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin IgG followed by AAT's Cy3® goat anti-mouse IgG (H&L) (Red, Left, Cat# 16862) or Vendor A's goat anti-mouse IgG conjugated withCy3® (Right). Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

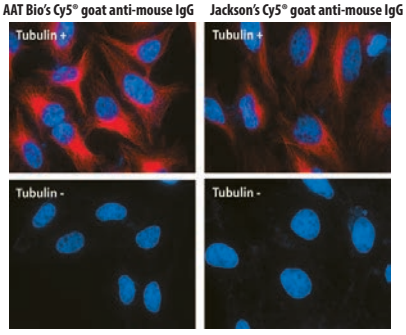

Figure 1.24 Image comparison of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by AAT's Cy5® goat anti-mouse IgG (H&L) (Red, Left, Cat# 16863) or Vendor A's Cy5® goat anti-mouse IgG conjugate (Red, Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

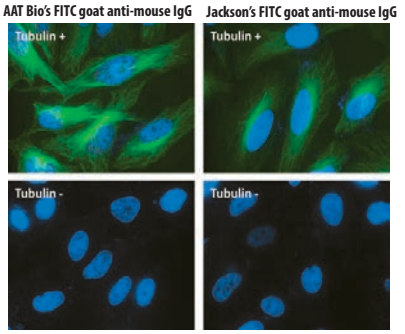

Figure 1.25 Image comparison of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by AAT's FITC goat anti-mouse IgG (H&L) (Green, Left, Cat# 16860) or Vendor A's FITC goat anti-mouse IgG conjugate (Green, Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

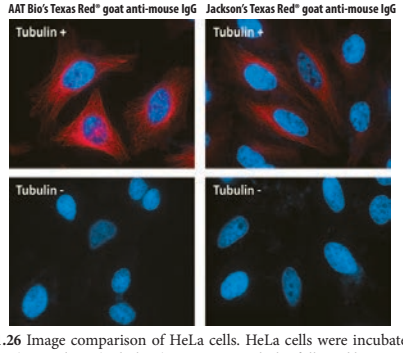

Figure 1.26 Image comparison of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by AAT's Texas Red® goat anti-mouse IgG (H&L) (Red, Left, Cat# 16861) or Vendor A's Texas Red® goat anti-mouse IgG conjugate (Red, Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

Classic Dye-Labeled Secondary Antibody Conjugates

AAT Bioquest offers an array of classic-dye labeled secondary antibody conjugates useful for applications such as the exploitation of FITC's high rate of photobleaching or pH sensitivity. These classic dyes are conjugated to secondary antibodies utilizing our proprietary Buccutite™ linking technology which significantly reduces the quenching effect of fluorophore tags by electron-rich amino acids of antibodies such as tryptophan, tyrosine and histidine residues. Traditional fluorescent label dyes and other classic dye-labels now have superior iFluor™ dye replacements. These alternatives have improved fluorescent intensity, greater photostability and a broader optimal pH range.

Biotinylated Secondary Antibody Conjugates

Biotin is a small and stable, water-soluble molecule that possesses a strong affinity for streptavidin. Exploitation of this interaction has been used to develop effective detection strategies for use in highly sensitive immunoassay applications. Biotin's small size allows for multiple molecules to be conjugated to a secondary antibody without compromising or impeding the function of the labeled antibody, a process known as biotinylation. Biotinylated secondary antibody conjugates provide a means for signal amplification which is ideal when detecting antigens expressed at relatively low levels.

AAT Bioquest's biotinylated secondary anti-mouse and anti-rabbit IgGs are conjugated to ensure that the optimal number of biotin molecules are labeled without impeding the functionality or affinity of the antibody. Our biotinylated secondary antibodies can be used for tissue and cell staining, ELISA, and other various immunoassays. For most assays, the biotin-labeled secondary antibody is added first, followed by the streptavidin labeled conjugate. For labeling proteins such as antibodies, use our ReadiView™ biotin products which have been optimized to produce robust and sensitive biotin probes for secondary detection methods. Furthermore, AAT Bioquest offers a convenient and robust ReadiLink™ Protein Biotinylation Kit for conjugating biotin to an antibody or a protein of choice. This kit includes a proprietary biotin succinimidyl ester which carries a color tag to indicate the degree of biotinylation, thus eliminating the troublesome HABA biotinylation determination step. The color tag is delicately selected to avoid interferences caused by either the binding of biotin or fluorescence detection. This kit is optimized for three conjugation reactions and includes all the necessary components for labeling and purifying antibodies.

Enzyme-Labeled Secondary Antibody Conjugates

The two most commonly used reporter enzymes, horseradish peroxidase (HRP) and alkaline phosphatase (ALP), are conjugated to secondary antibodies as detection

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agents in immunoassay applications such as ELISA and immunohistochemistry (IHC). These enzymes produce a visual and measurable chromogenic, fluorogenic or luminescent signal when reacted with their appropriate soluble organic substrate. A key advantage of enzyme-labeled secondary antibody conjugates is its capacity to amplify the primary signal by converting many molecules of its respective substrate. AAT Bioquest offers cross-adsorbed and highly purified IgGs that are conjugated to HRP or ALP enzymes with our proprietary Buccutite™ protein crosslinking technology. Buccutite™ technology enables HRP and ALP to be readily conjugated to antibodies in neutral conditions producing a high yield of enzyme-labeled antibody conjugates. These conjugates retain full functionality of both their IgG and reporter enzyme components, and are ideally suited for ELISA assays.

Alexa Fluor®-Labeled Secondary Antibody Conjugates

AAT Bioquest offers a small collection of fluorescently labeled probes consisting of goat anti-mouse and goat anti-rabbit IgG secondary antibodies conjugated with either Alexa Fluor® 350, 488 or 594 dyes (Alexa Fluor® is the trademark of ThermoFisher). Alexa Fluor® 350, 488 and 594 labeled-secondary antibody conjugates are prepared by reacting cross-adsorbed goat anti-mouse or goat anti-rabbit IgG whole antibodies with its respective Alexa Fluor® 350, 488 or 594 NHS ester. Prior to conjugation, each goat-anti mouse and goat anti-rabbit IgG whole antibodies have been cross-adsorbed against human serum and human IgG to minimize cross-reactivity. Alexa Fluor®-labeled conjugates have typically 4-6 fluorophores per IgG molecule. These fluorescent secondary antibody conjugates are useful in detection, sorting or purification of specific targets and are ideal for fluorescence microscopy and confocal laser scanning microscopy, flow cytometry, and fluorescent Western Blot detection.

AAT Bioquest's Custom Antibody Conjugation Services

AAT Bioquest offers same-day custom conjugation of antibodies or proteins with a wide array of labels, such as biotin, HRP and over 20 fluorophores. Our service is designed for high-quality results with fast turnaround, to supply scientists with flexible tools that help expand their research.

Features of AAT Bioquest's custom antibody conjugation services include:

- Guaranteed quality with 95% purity rating
- Affordable price tiers that work with your budget
- Scalable service with minimum order of 50 µg
- Same day order fulfilment means your conjugate ships the day you order it

You may supply your own antibody or chose from 3000+ monoclonal and polyclonal antibodies in our catalog. Contact us for a quote today, and let us put our expertise to work for you!

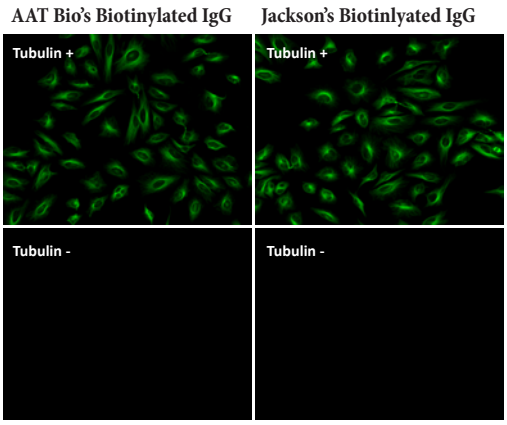

Figure 1.27 Image comparison of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by AAT's biotinylated goat anti-mouse IgG (H&L) (Left, Cat# 16729) or Jackson's biotinylated goat anti-mouse IgG conjugate (Right). At the end, cells were incubated with AAT's iFluor™488-streptavidin conjugate (Cat# 16955).

Custom Antibody Conjugation Services

How Does it Work?

Antibody conjugates allow for the study of specific antigens in biological systems. Antibodies can be conjugated with reporter enzymes and with a variety of fluorochromes for use in applications such as Western Blot, ELISA, and flow cytometry.

Picking the Right Label

Picking the correct label to use for your experiment can be difficult, especially when there are so many choices. Here are some considerations to keep in mind:

Single/Multiplex Format:

- **Explanation:** If you are running a multiplex experiment, be aware of spectral overlaps. Too much overlap will lead to signal bleed-through between fluorophores.
- **Our Suggestion:** Use our Spectrum Viewer web application to compare fluorophore excitation/emission and minimize overlaps.

Instrumentation:

- **Explanation:** Make sure that your instrument can properly detect the chosen fluorophore. Is it colorimetric or fluorimetric? Does it have the proper excitation sources? Does it have the correct filters?
- **Our Suggestion:** Check our fluorophore product page for specific spectral properties to see if it is compatible with your instrumentation.

Experimental Conditions:

- **Explanation:** Does your experiment require special conditions such as temperature or pH? Many fluorophores are sensitive to experimental conditions, which will impact their brightness and susceptibility to photo-bleaching.
- **Our Suggestion:** Check our fluorophore product page for specific restrictions on usage.

Available Labels

AAT Bioquest offers a comprehensive list of fluorophores, reporter enzymes, tandem dyes and biotin for custom conjugations.

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Product Ordering Information For Classic Dye, Biotinylated, Enzyme and Alexa Fluor®-Labeled Secondary Antibody Conjugates

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
16380	AF350 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	346	442
16395	AF350 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	346	442
16383	AF488 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	490	525
16398	AF488 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	490	525
16388	AF594 goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	590	617
16404	AF594 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	590	617
16729	Biotinylated goat anti-mouse IgG (H+L)	1 mg	n/a	n/a
16794	Biotinylated goat anti-rabbit IgG (H+L)	1 mg	n/a	n/a
16854	Cy3® goat anti-mouse IgG (H+L)	1 mg	555	565
16862	Cy3® goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	555	565
16870	Cy3® goat anti-rabbit IgG (H+L)	1 mg	555	565
16878	Cy3® goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	555	565
16855	Cy5® goat anti-mouse IgG (H+L)	1 mg	649	665
16863	Cy5® goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	649	665
16871	Cy5® goat anti-rabbit IgG (H+L)	1 mg	649	665
16879	Cy5® goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	649	665
16856	Cy7® goat anti-mouse IgG (H+L)	1 mg	749	776
16864	Cy7® goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	749	776
16872	Cy7® goat anti-rabbit IgG (H+L)	1 mg	749	776
16880	Cy7® goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	749	776
16852	FITC goat anti-mouse IgG (H+L)	1 mg	492	515
16860	FITC goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	492	515
16868	FITC goat anti-rabbit IgG (H+L)	1 mg	492	515
16876	FITC goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	492	515
16728	HRP-labeled goat anti-mouse IgG (H+L)	1 mg	n/a	n/a
16793	HRP-labeled goat anti-rabbit IgG (H+L)	1 mg	n/a	n/a
16853	Texas Red® goat anti-mouse IgG (H+L)	1 mg	582	602
16861	Texas Red® goat anti-mouse IgG (H+L) *Cross Adsorbed*	1 mg	582	602
16869	Texas Red® goat anti-rabbit IgG (H+L)	1 mg	582	602
16877	Texas Red® goat anti-rabbit IgG (H+L) *Cross Adsorbed*	1 mg	582	602

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FLUORESCENT STREPTAVIDIN CONJUGATES

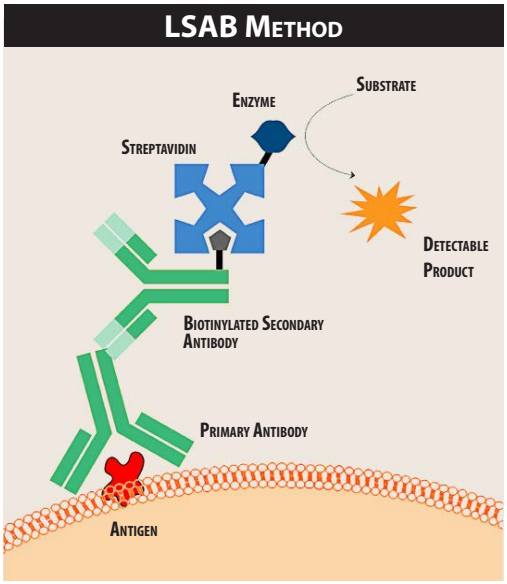

Figure 2.1 Labeled Streptavidin Biotin (LSAB) Method

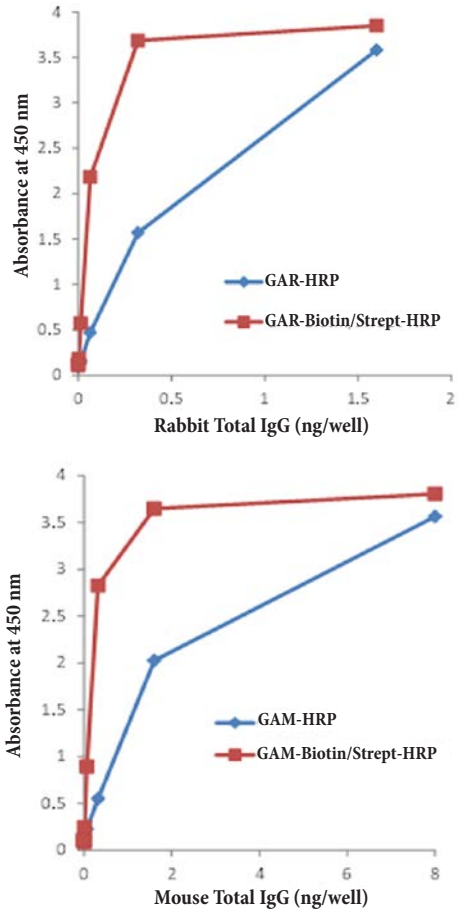

Figure 2.2 Sensitivity enhancement with biotinylated goat anti-mouse IgG and the streptavidin-HRP conjugate. Plates are coated with indicated concentrations of rabbit (Top), or mouse (Bottom) IgG. The amount of coated IgG is detected directly with goat anti-rabbit or goat anti-mouse IgG HRP conjugates, or with biotinylated goat anti-rabbit or goat anti-mouse IgG followed by the streptavidin-HRP conjugate.

Introduction

Streptavidin is a deglycosylated, 52.8 kD avidin protein purified from the bacterium *Streptomyces avidinii*. Its near-neutral isoelectric point reduces potential background interference from nonspecific interactions with negatively charged cell surfaces and nucleic acids that may occur when using positively charged proteins such as avidin. Streptavidin has an extraordinarily high binding affinity for biotin with a dissociation constant (Kd) of ~10^{-14} mol/L, making biotin-streptavidin bonds one of the strongest non-covalent interactions in nature.

The strong affinitive properties of biotin and streptavidin's interactions are exploited in immunoassay applications for developing versatile and sensitive detection systems. Streptavidin-biotin complexes exhibit resistance to organic solvents, proteolytic enzymes and extreme temperatures and pHs. Each streptavidin molecule has the capacity to bind four biotin molecules with high selectivity and affinity. Streptavidin's multiple binding sites promotes amplification of the primary signal making this a powerful secondary detection reagent. This allows for a more sensitive and accurate detection of low-level target analytes expressed in a complex biological sample.

Streptavidin-biotin complexes are commonly exploited through the Labeled Streptavidin Biotin (LSAB) method, which is an indirect detection procedure. The LSAB method utilizes an unlabeled primary antibody that is directed against the target of interest, which in turn is subsequently detected by a biotinylated secondary antibody. This secondary antibody is typically directed against the immunoglobulin class or subclass of the primary antibody's species. Lastly, either a dye-labeled streptavidin conjugate is used to detect and produce a fluorescent signal or an enzyme-labeled streptavidin conjugate in tandem with its appropriate substrate is used to generate a chromogenic signal. Compared to the Avidin Biotin Complex (ABC), the smaller size of the LSAB complex allows for better penetration in tissue samples making LSAB a superior alternative. Additionally, the LSAB method has been noted to reduce background interference and improve the sensitivity of detection. (Refer to Figure 2.1 for the detection mechanism of the LSAB method)

AAT Bioquest offers a comprehensive selection of streptavidin conjugates labeled with our superior line of iFluor™ and mFluor™ dyes, classic-dyes, phycobiliproteins and reporter enzymes. Our streptavidin conjugates have been optimized for applications involving immunofluorescence microscopy, flow cytometry, ELISA and Western Blot. In conjunction with biotinylated goat anti-mouse IgG, the streptavidin-HRP conjugate reveals a 10-fold sensitivity increase in response to mouse IgG in ELISA (Figure 2.2). AAT Bioquest's iFluor™ streptavidin conjugates are optimized with the best dye to streptavidin ratio for immunofluorescence microscopy and flow cytometry. Our enzyme-labeled streptavidin conjugates are prepared for the highest sensitivity for use in direct and indirect ELISA assays.

Fluorescent Streptavidin Conjugates

Fluorescent-labeled streptavidin conjugates are widely utilized in multi-color analysis and in an array of applications including immunofluorescence, immunocytochemistry and flow-cytometry. AAT Bioquest offers a comprehensive selection of streptavidin

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conjugates labeled with our superior iFluor™ dyes and with classic fluorescent labeling dyes such as the Cy® dyes, FITC and Texas Red®. When choosing the appropriate fluorescent labeled streptavidin conjugate for your desired application, consider these following factors:

- Select the brightest fluorescent dye available for your instrument.
- Select fluorescent labels with minimal fluorescent overlap.
- For samples which autofluoresce, select streptavidin conjugates which emit light from a different channel than your autofluorescing sample.

iFluor™ Streptavidin Conjugates

AAT Bioquest's extensive line of iFluor™-labeled streptavidin conjugates span the entire spectrum from UV to infra-red light producing intense fluorescent signals with greater photostability and minimal quenching effect when coupled to proteins. Each iFluor™-labeled streptavidin conjugate has been optimized with the best dye to streptavidin ratio for superior detectability in immunofluorescence microscopy and flow cytometry. Data from microscopic and flow cytometric analysis demonstrates the superiority of iFluor™-labeled streptavidin conjugates compared to classic dye labeled-streptavidin conjugates and fluorescently labeled-streptavidin conjugates from leading suppliers.

AAT Bioquest's iFluor™-labeled streptavidin conjugates are high quality reagents that are extensively tested to help ensure bright staining with minimal background interference. The iFluor™-labeled streptavidin conjugates have the same following features as our iFluor™-labeled secondary antibody conjugates. (Table 2)

Benefits of iFluor™ Streptavidin Conjugates:

- **Bright Dyes:** the fluorescence intensity of iFluor™ dyes outperform or match other spectrally similar dyes (e.g., Alexa Fluor®)
- **Great Photostability:** Increased photostability allows for longer periods of image capture
- **pH Insensitivity:** iFluor™ fluorescence intensity remains high over a broad pH range
- **Good Water Solubility:** Good water solubility prevents the precipitation and aggregation of iFluor™ conjugated antibodies
- **Optimized & Validated Performance:** Optimized for immunofluorescence microscopy and flow cytometry

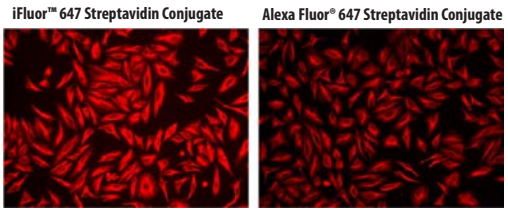

Figure 2.3 Image comparison of HeLa cells. HeLa cells were incubated with mouse anti-tubulin and biotin goat anti-mouse IgG followed by AAT's iFluor™ 647-streptavidin conjugate (Red, Left, Cat# 16966) or streptavidin conjugated with Alexa Fluor® 647 (Red, Right), respectively.

Table 2. The spectral properties of iFluor™ streptavidin conjugates

Fluorophore	Replaces	Excitation (nm)	Emission (nm)
iFluor™ 350	Alexa Fluor® 350, DyLight™ 350	345	442
iFluor™ 405	Alexa Fluor® 405, DyLight™ 405	401	420
iFluor™ 488	Alexa Fluor® 488, DyLight™ 488	491	514
iFluor™ 514	Alexa Fluor® 514	518	542
iFluor™ 532	Alexa Fluor® 532	531	556
iFluor™ 546	Alexa Fluor® 546	541	557
iFluor™ 555	Alexa Fluor® 555, DyLight™ 550, Cy3®, TRITC	555	565
iFluor™ 568	Alexa Fluor® 568	568	587
iFluor™ 594	Alexa Fluor® 594, DyLight™ 594, Texas Red®	594	614
iFluor™ 610	Alexa Fluor® 610	605	627
iFluor™ 633	Alexa Fluor® 633, DyLight™ 633	638	655
iFluor™ 647	Alexa Fluor® 647, DyLight™ 650, Cy5®	649	665
iFluor™ 680	Alexa Fluor® 680, DyLight™ 680, Cy5.5®, IRDye® 700	676	695
iFluor™ 700	Alexa Fluor® 700	685	710
iFluor™ 750	Alexa Fluor® 750, DyLight™ 750, Cy7®	749	775
iFluor™ 790	Alexa Fluor® 790, DyLight™ 800, IRDye® 800	782	811
iFluor™ 810	No other commercial equivalents	809	821
iFluor™ 820	No other commercial equivalents	820	825
iFluor™ 830	No other commercial equivalents	832	838
iFluor™ 860	No other commercial equivalents	863	868

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Table 3. mFluor™ Dyes

If You Are Using	Try This mFluor™ Dye
Pacific Blue	mFluor™ Violet 450
AmCyan	mFluor™ Violet 510
Pacific Orange or Krome Orange	mFluor™ Violet 540
RPE	mFluor™ Blue 570
APC-Cy5.5®, APC-Alexa Fluor® 680 or APC-Alexa Fluor® 700 tandem	mFluor™ 700
APC-Cy7®, APC-Alexa Fluor® 750 or APC-H7 tandem	mFluor™ 780

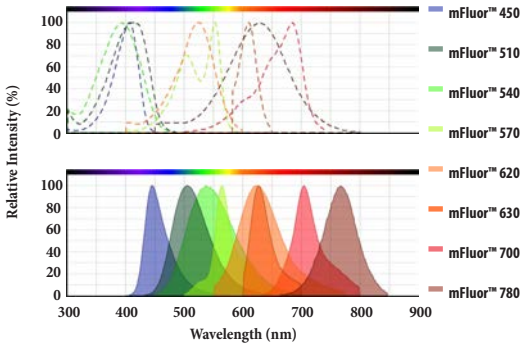

Figure 2.4 Comprehensive excitation (above) and emission (below) spectra of the mFluor™ dyes series.

mFluor™ Streptavidin Conjugates Optimized For Flow Cytometry

AAT Bioquest provides a series of excellent fluorescent labeling mFluor™ dyes that span the entire UV-visible spectrum. Each mFluor™ dye has been optimized to have its maximum absorption match one of the major lasers typically equipped in a flow cytometer (Table 4). For example, all of our mFluor™ violet dyes conjugated to streptavidin have been optimized to have their excitations close to the 405 nm violet laser line. mFluor™-streptavdin conjugates are excellent alternatives to the phycobiliprotein-based tandems which prove quite difficult when coupling to an antibody or other biomolecules (Table 3).

AAT Bioquest's mFluor™-labeled streptavidin conjugates boast a much larger Stokes Shifts when compared to our iFluor™ conjugates, while still retaining the same signal intensity, photostability and quenching characteristics as the iFluor™ dyes. mFluor™-streptavidin conjugates are robust and highly fluorescent over a broad pH range with little pH-sensitivity. The capacity of mFluor™ dyes to produce a larger Stokes Shift makes them ideally suited for secondary detection applications involving multicolor flow cytometric analysis.

In conjunction with biotinylated primary antibodies, you can choose the appropriate mFluor™-labeled streptavidin conjugate for sensitive flow cytometric detection following the guideline summarized in the following table.

Table 4. mFluor™ streptavidin conjugates and their corresponding laser

Excitation	Blue	Green	Orange	Red	Deep Red	NIR
Violet laser (405 nm)	mFluor™ Violet 430 mFluor™ Violet 450	mFluor™ Violet 510	mFluor™ Violet 570	mFluor™ Violet 605	mFluor™ Violet 650 mFluor™ Violet 710	mFluor™ Violet 780
Argon laser (488 nm)			mFluor™ Blue 570			
Green Laser (532 nm)				mFluor™ Green 620		
Yellow laser (561 nm)				mFluor™ Yellow 630		
He-Ne laser (633 nm)					mFluor™ Red 700	mFluor™ Red 780
Red laser (647 nm)					mFluor™ Red 700	mFluor™ Red 780

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The Classic-Dye Labeled Streptavidin Conjugates

Traditional fluorescent label dyes such as the Cy® dye series, FITC, Texas Red® and other classic dye-labels now have superior alternative iFluor™ and mFluor™ dye replacements with improved fluorescent intensity, greater photostability and a broader optimal pH range. However, traditional dyes are still useful in experiments which exploit their photobleaching properties and pH-sensitivity. AAT Bioquest offers a line of classic-dye labeled streptavidin conjugates that are significantly brighter and more sensitive than the equivalent labeled streptavidin conjugates available from other commercial vendors. This is because these classic-dyes and fluorophores are conjugated to streptavidin utilziing our proprietary Buccutite™ linking technology, which significantly reduces the quenching effect of fluorophore tags by electron-rich amino acids of streptavidin. Under the same conditions, our classic dye-labeled streptavidin conjugates are significantly more sensitive than similar streptavidin conjugates form other commercial sources.

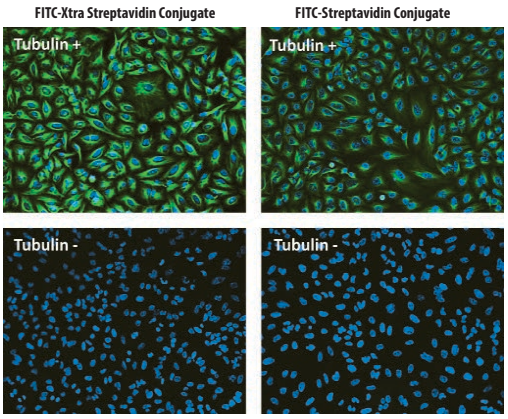

Figure 2.5 Image comparison of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin and biotin goat anti-mouse IgG followed by FITC-Xtra streptavidin conjugate (Green, Left, Cat# 135) or FITC-streptavidin conjugate (Green, Right, Cat# 16910), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17530).

trFluor™ Streptavidin Conjugates: Optimized For Time-Resolved Fluorescence-Based Assays

AAT Bioquest's trFluor™ streptavidin conjugates are comprised of streptavidin, as the biotin-binding protein, with trFluor™ Eu or trFluor™ Tb covalently attached as the time-resolved red fluorescent europium (Eu) label or green fluorescent terbium (Tb) label (Figure 2.7). trFluor™ streptavidin conjugates are commonly used in conjunction with biotinylated secondary antibodies as a secondary detection reagent for indirect immunofluorescent staining. They are very valuable tools for biotin-streptavidin based biological assays and tests which utilize a TR-FRET platform (Table 5).

Key Feature of trFluor™ Dyes:

- No fluoride addition or enhancing solution is required.
- Available in a variety of reactive forms.
- Easier to be conjugated to biomolecules with a higher conjugation yield than other TRF dyes.
- Maximally excited by the common light sources at ~350 nm.

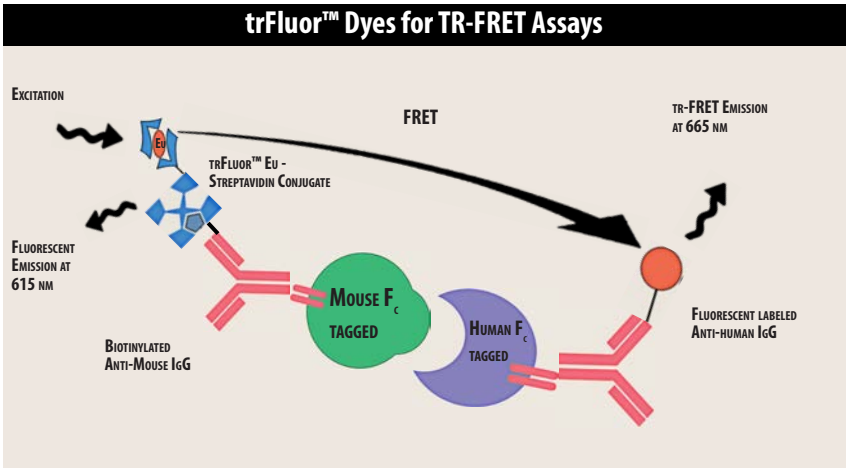

Figure 2.7. TR-FRET Assay Principle using trFluor™-labeled streptavidin conjugates

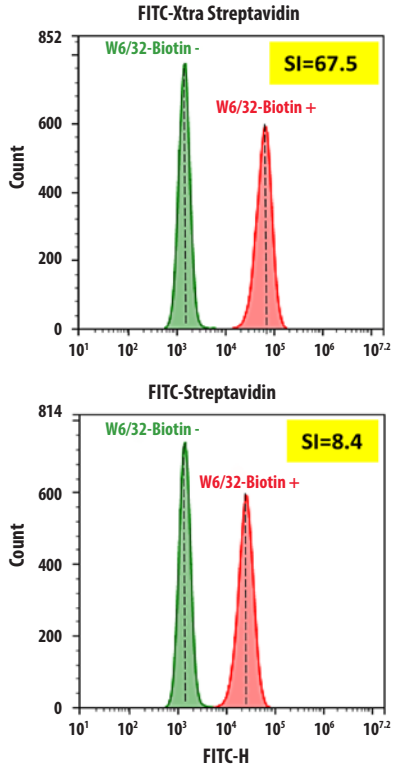

Figure 2.6 HL-60 cells were incubated with (Red, +) or without (Green, -) mouse anti-HLA-ABC (W6/32 mAb) and biotin goat anti-mouse IgG followed by FITC-Xtra streptavidin conjugate (Cat# 135) or FITC-streptavidin conjugate (Cat# 16910), respectively. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in FITC channel. The stain index (SI) of each conjugate was calculated.

Table 5. Typical acceptors for the time -resolved luminescent probes

trFluor™ Donors	Recommended Acceptors
trFluor™ Eu	iFluor™ 647, TF5, APC
trFluor™ Tb	iFluor™ 488, TF2, FITC

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Product Ordering Information For iFlour™, mFluor™, trFluor™ and Classic-Dye Labeled-Streptavidin Conjugates

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
16912	Cy3®-streptavidin conjugate	1 mg	555	565
16913	Cy5®-streptavidin conjugate	1 mg	649	665
16914	Cy7®-streptavidin conjugate	1 mg	749	776
16910	FITC-streptavidin conjugate	1 mg	492	515
135	FITC-Xtra streptavidin conjugate	25 mg	494	520
136	FITC-Xtra streptavidin conjugate	100 mg	494	520
16950	iFluor™ 350-streptavidin conjugate	200 µg	345	442
16980	iFluor™ 350-streptavidin conjugate	1 mg	345	442
16952	iFluor™ 405-streptavidin conjugate	200 µg	401	420
16982	iFluor™ 405-streptavidin conjugate	1 mg	401	420
16955	iFluor™ 488-streptavidin conjugate	200 µg	491	514
16985	iFluor™ 488-streptavidin conjugate	1 mg	491	514
16956	iFluor™ 514-streptavidin conjugate	200 µg	518	542
16986	iFluor™ 514-streptavidin conjugate	1 mg	518	542
16957	iFluor™ 532-streptavidin conjugate	200 µg	531	556
16987	iFluor™ 532-streptavidin conjugate	1 mg	531	556
16958	iFluor™ 546-streptavidin conjugate	200 µg	541	557
16959	iFluor™ 555-streptavidin conjugate	200 µg	559	569
16989	iFluor™ 555-streptavidin conjugate	1 mg	559	569
16960	iFluor™ 568-streptavidin conjugate	200 µg	568	587
16962	iFluor™ 594-streptavidin conjugate	200 µg	592	614
16992	iFluor™ 594-streptavidin conjugate	1 mg	592	614
16965	iFluor™ 633-streptavidin conjugate	200 µg	638	655
16995	iFluor™ 633-streptavidin conjugate	1 mg	638	655
16966	iFluor™ 647-streptavidin conjugate	200 µg	654	674
16996	iFluor™ 647-streptavidin conjugate	1 mg	654	674
16968	iFluor™ 680-streptavidin conjugate	200 µg	682	701
16997	iFluor™ 680-streptavidin conjugate	1 mg	682	701
16970	iFluor™ 700-streptavidin conjugate	200 µg	693	713
16998	iFluor™ 700-streptavidin conjugate	1 mg	693	713
16973	iFluor™ 750-streptavidin conjugate	200 µg	753	779
16999	iFluor™ 750-streptavidin conjugate	1 mg	753	779
16935	mFluor™ Blue 570-streptavidin conjugate	100 µg	553	570
16938	mFluor™ Green 620-streptavidin conjugate	100 µg	522	617
16946	mFluor™ Red 700-streptavidin conjugate	100 µg	657	700
16948	mFluor™ Red 780-streptavidin conjugate	100 µg	629	780
16930	mFluor™ Violet 450-streptavidin conjugate	100 µg	403	454
16931	mFluor™ Violet 510-streptavidin conjugate	100 µg	414	508
16932	mFluor™ Violet 540-streptavidin conjugate	100 µg	399	540
16927	mFluor™ Violet 570-streptavidin conjugate	100 µg	408	571
16928	mFluor™ Violet 650-streptavidin conjugate	100 µg	408	655
16929	mFluor™ Violet 710-streptavidin conjugate	100 µg	408	708
16934	mFluor™ Violet 780-streptavidin conjugate	100 µg	408	779
16942	mFluor™ Yellow 630-streptavidin conjugate	100 µg	611	630
16911	Texas Red®-streptavidin conjugate	1 mg	582	602
16925	trFluor™ Eu-streptavidin conjugate	100 µg	346	617
16926	trFluor™ Tb-streptavidin conjugate	100 µg	330	544

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Enzyme-Labeled Secondary Detection Conjugates

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HRP Detection Kit Key Features:

- **Broad Application:** Can be used for quantifying HRP activities in solutions and solid surfaces (e.g, ELISA).
- **Sensitive:** Detect as low as 100 µU/mL of HRP in solution.
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time. No wash is required.
- **Non-Radioactive:** No special requirements for waste treatment.

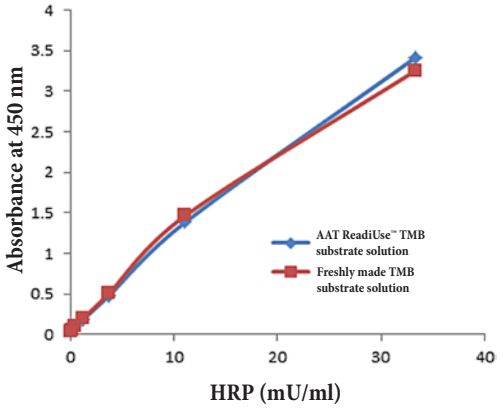

Figure 3.1 The performance comparison of AAT ReadiUse™ TMB substrate solution (Blue Line) with the freshly made TMB substrate solution (Red Line). The ReadiUse™ TMB and freshly made TMB solutions show the identical sensitivity in detecting HRP.

Detection of Horse Radish Peroxidase and Its Conjugates

Horseradish peroxidase (HRP) is a 44 kD glycoprotein that functions optimally at a near-neutral pH and serves as an ideal tag enzyme in immunoassays. It is a heme-containing oxidoreductase which catalyzes the reductive cleavage of hydrogen peroxide by an electron donor. HRP is comprised of six lysine residues which can be conjugated to a biological molecule of interest to determine the presence of a target analyte. For example, one form of secondary detection involves conjugating HRP to an antibody for the detection of a target analyte in ELISA assay. The antibody functions as a highly sensitive probe detecting the target analyte, while the HRP enzyme reacts with its appropriate substrate to generate a detectable and measurable signal.

HRP is ideal for these applications because it is less expensive, smaller in size and more stable than other alternative tag enzymes such as alkaline phosphatase (ALP). Due to its small size, HRP rarely causes steric hindrance problems with the antibody-antigen complex. It exhibits a high turnover rate that permits the generation of strong detection signals in a relatively short duration of time. HRP produces a chromogenic, fluorimetric, or luminescent product of the labeled molecule when incubated with a proper substrate, allowing it to be readily detected and quantified. HRP conjugates are commonly used in techniques such as Western Blotting, ELISA and immunohistochemistry due to its monomeric nature and the ease with which it produces optically detectable products. AAT Bioquest offers one of the largest collection of HRP substrates and assay kits that are used for HRP detection and conjugations. Our kits have been optimized to include all the necessary components and substrates to perform colorimetric, fluorimetric and chemiluminescent assays.

Colorimetric Detection of HRP and Its Conjugates

AAT Bioquest offers two substrate solutions that have been optimized for colorimetric assays with HRP conjugates and hydrogen peroxide (H_2O_2) in microwell plates or test tubes. Our ReadiUse™ TMB Substrate Solution is the most convenient ready-to-use HRP substrate solution that is pre-mixed with TMB and hydrogen peroxide. Our TMB solution allows the following HRP reaction kinetics to be readily followed:

$$TMB + H_2O_2 \xrightarrow{HRP} TMB^+ + H_2O \quad (\lambda_{max} = 650\ nm)$$

It is highly stable and sensitive at detecting HRP activity, yielding an oxidized blue product that becomes yellow upon stopping with 1N HCl. Use of a stop solution enhances sensitivity 2 to 4-fold and allows the resulting yellow solution to be read at a wavelength of 450 nm. ReadiUse™ TMB Substrate Solution provides an ultrasensitve quantitative substrate system detecting as little as 20 picograms of mouse IgG when used in conjunction with AAT Bioquest's HRP labeled goat anti-mouse IgG conjugate (Figure 3.1).

Our ReadiUse™ ABTS is another optimized HRP substrate solution which allows the

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HRP reaction to be completed in a single addition step. Our ABTS solution allows for the following HRP reaction kinetics to be followed:

$$ABTS + H_2O_2 \xrightarrow{HRP} ABTS^{+} + H_2O$$
(λ_{max} = 405 nm) (λ_{max} = 420 nm)

HRP and HRP conjugates facilitate the ABTS oxidation in the presence of H_2O_2, turning ABTS into its blue-green oxidized product. The oxidized ABTS product has a maximum absorption at 420 nm which can easily be monitored by a spectrophotometer. The ReadiUse™ ABTS solution allows for the continuous measurement of enzyme activity without any need for stopping, and has been optimized for ELISA assays with HRP conjugates.

Fluorimetric Detection of HRP and Its Conjugates

AAT Bioquest offers an extensive line of highly sensitive, proprietary fluorogenic peroxidase substrates excellent for fluorimetric assays with HRP conjugates and H_2O_2. Our Amplite™ Blue peroxidase substrate is a soluble, fluorogenic substrate used for the detection of peroxidase activity. It allows rapid HRP detection assays to be performed with greater sensitivity than the colorimetric HRP substrates such as TMB, ABTS and OPD. Amplite™ Blue generates a highly fluorescent material that has a maximum absorption at 664 nm. This near infrared absorption minimizes impeding background interference often caused by the auto-fluorescence of biological samples resulting in high signal-to-noise ratios. Our Amplite™ Red HRP substrate has virtually identical properties as our Amplite™ Blue except that it generates a highly red fluorescent product with has a max absorption at 571 nm and a max emission at 585 nm.

AAT Bioquest's Amplite™ ADHP is chemically and spectrally identical to Amplex® Red. Amplite™ ADHP is a sensitive fluorogenic peroxidase substrate that has a much lower background than similar substrates from other commercial vendors. It generates highly fluorescent resorufin that has a max absorption at 571 nm and max emission at 585 nm with minimal air-oxidation of ADHP. To this date ADHP has been known to be one of the most sensitive and stable fluorogenic probes for detecting HRP and H_2O_2. ADHP is frequently used to detect HRP in many immunoassays, and can also be used to detect trace amounts of H_2O_2. The ADHP-based hydrogen peroxide detection system is at least one order of magnitude more sensitive than the commonly used scopoletin assay for H_2O_2. Since H_2O_2 is produced in many enzymatic redox reactions, ADHP can be used in coupled enzymatic reactions to detect the activity of many oxidases and other related enzymes or substrates.

Our Amplite™ IR is a water soluble fluorogenic peroxidase substrate that generates a near infrared fluorescence upon reacting with peroxidase and H_2O_2. It can be used to detect both hydrogen peroxide and HRP. Amplite™ IR generates a product that has a max absorption at 647 nm and a max emission at 670 nm. This near infrared absorption and fluorescence minimizes the assay background interference that is often caused by the auto-absorption or autofluorescence of biological samples that rarely absorb light beyond 600 nm.

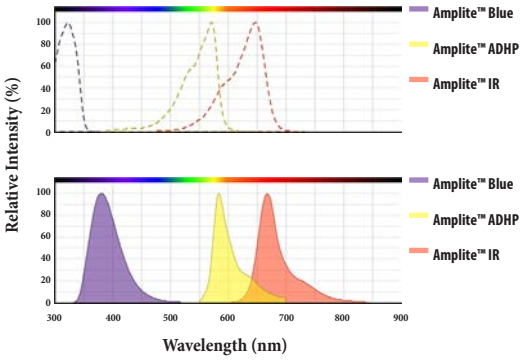

Figure 3.2 Excitation (above) and emission (below) spectra of Amplite™ Blue (Violet, Cat# 11005), Amplite™ ADHP (Yellow Cat# 11000) and Amplite™ IR (Red, Cat# 11009).

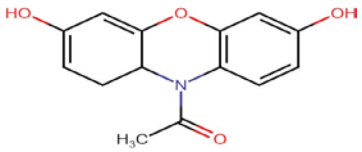

Figure 3.3 Image of the chemical structure of Amplite™ ADHP (Cat# 11000).

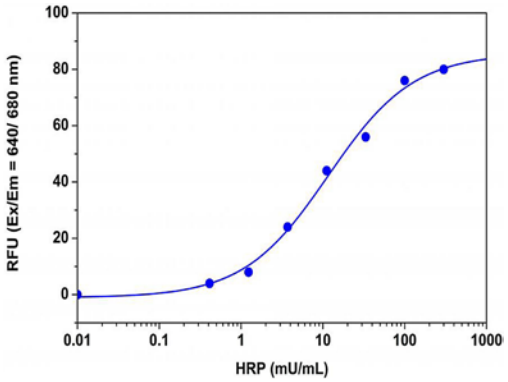

Figure 3.4 HRP dose response was measured with Amplite™ Fluorimetric Peroxidase Assay Kit in a solid black NIR (Cat# 11553) 384-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

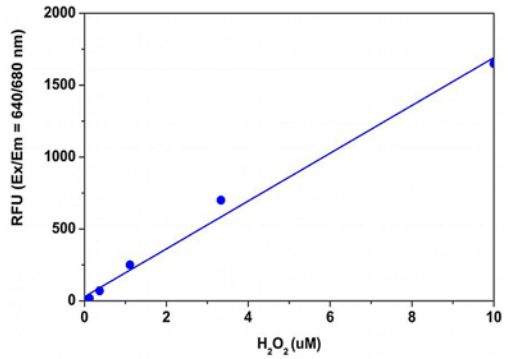

Figure 3.5 H_2O_2 dose response was measured in a solid black 96-well plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit using Amplite™ IR substrate (Cat# 11009) .

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Amplex® Red vs. Amplite™ IR

Excitation/Emission (nm):

- **Amplex® Red :** 571 / 585
- **Amplite™ IR :** 647 / 670

Advantage Amplite™ IR: The 647 nm excitation of Amplite™ IR perfectly match the laser lines of He-Ne, red diode and Krypton, which are equipped with most of fluorescence instruments. The longer excitation and emission wavelengths of Amplite™ IR significantly reduce the sample background resulted from the autofluorescence of samples.

Water Solubility:

- **Amplex® Red :** Extremely Low
- **Amplite™ IR :** High

Advantage Amplite IR: Amplex Red® must be dissolved in DMSO while the aqueous solution of Amplite™ IR can be readily made.

Working pH Range:

- **Amplex® Red :** ≥ 7.0
- **Amplite IR :** 4-11

Advantage Amplite™ IR: Resorufin, the HRP-derived product of Amplex® Red, only gives its maximum fluorescence under basic conditions. In acidic solutions resorufin is non-fluorescent.

Over Radiation:

- **Amplex® Red :** Severly in some cases
- **Amplite™ IR :** Minimal

Advantage Amplite™ IR: Amplex® Red can be over oxidized to the much less fluorescent resazurin. Our Signal Guard™ HRP reaction stopping solution is designed for use in conjunction with Amplex® Red and Amplex®UltraRed HRP substrates. Under the same conditions, our Signal Guard™ HRP reaction stopping solution significantly outperforms the Amplex® Red/UltraRed Stop Reagent (#A33855) from ThermoFisher.

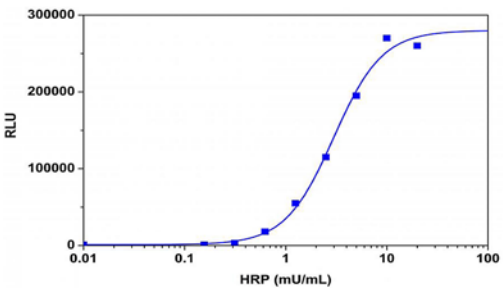

Figure 3.6 HRP dose responses were measured with Amplite™ Luminometric Peroxidase Assay Kit (Cat# 11559) in a 384 - well black plate. As low as 150 mU/mL of peroxidase can be detected with 30 minutes incubation time (n=3).

Compared to the other HRP substrates such as dihydrofluoresceins and dihydrorhodamines, the air-oxidation of Amplite™ IR is minimal. Amplite™ IR generates a fluorescence that is pH-independent ranging from pH 4-10 making it a superior alternative to Amplex® Red. Amplex® Red is susceptible to quenching during fluorimetric detections that require low pH. Amplite™ IR can also be used to detect trace amounts of hydrogen peroxide. Since H_2O_2 is produced in many enzymatic redox reactions, Amplite™ IR can be used in coupled enzymatic reactions to detect the activity of many oxidases and other related enzymes or substrates.

Fluorogenic HRP substrates are susceptible to over oxidation which significantly reduces their detection sensitivity. HRP reactions are typically performed in microplate wells by the addition of a fluorogenic HRP substrate, resulting in a continuous fluorescence change. Therefore, it is critical to ensure that the timing of the standard and unknown sample measurements is identical. Our Signal Guard™ HRP reaction stopping solution provides the convenience and control necessary to terminate the fluorescence signal-generating reaction at a user-determined time point. Addition of the stop reagent prevents the loss of catalytic activity and maintains the active conformation of the antibody portion of the conjugate, stabilizing the fluorescence signal. The Signal Guard HRP reaction stopping solution is designed for use in conjunction with ADHP, Amplite™ Red and Amplex® Ultra Red fluorogenic substrates. It allows for conjugates to be stored at a ready-to-use concentration without further dilution making repeated assays more easily reproducible.

Luminometric Detection of HRP and Its Conjugates

Enhanced chemiluminescence is a common technique for a variety of detection assays in biology. An HRP enzyme is tethered to a target analyte usually though labeling an immunoglobulin that selectively recognizes the analyte of interest. This enzyme complex catalyzes the conversion of the enhanced chemiluminescent substrate into a sensitized reagent in the vicinity of the molecule of interest. The further oxidation of the substrate by H_2O_2 produces an excited molecule which emits easily detectable light.

Amplite™ Luminometric Peroxidase Assay kit uses our Amplite™ luminometric HRP substrate to quantify peroxidase in solutions. It includes an optimized "mix and read" assay protocol and is sensitive at detecting concentrations as low as 100 µU/mL of HRP. This assay can be performed in a convenient 96-well or 384-well microtiter-plate format and can be easily measured by a luminescence microplate reader. Our Amplite™ Luminometric kit can be used for ELISA assays which focus on characterizing the kinetics of enzymatic reactions and high throughput screenings.

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Assay Kits For Detecting HRP and Its Conjugates

Amplite™ Colorimetric Peroxidase Assay Kit is a fast, one-step, homogenous HRP assay that can be used for quantifying HRP activities in solution or on a solid surface. This kit contains our ultrasensitive chromogenic HRP substrate, Amplite™ Blue, which is excellent at detecting concentrations as low as 3 µU/mL of HRP in solution. Amplite™ Blue generates a highly absorptive product that has a max absorption at 664 nm. This near infrared absorption minimizes the background absorption often caused by the auto-absorption of biological samples. The kit provides an optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and can easily be adapted to automation without a separation step. Its signal can be easily read by an absorbance microplate reader at 664 nm.

Amplite™ Fluorimetric Peroxidase Assay Kits are one-step, homogeneous, no wash assay systems that come packaged with either our Amplite™ IR substrate (Kit 11553) or our Amplite™ Red HRP substrate (Kit 11552) to quantify peroxidase in solutions. Both kits include an optimized and robust assay protocol that is compatible with HTS liquid handling instruments. They are excellent for ELISA assays characterizing kinetics of enzyme reactions and high throughput screenings. Fluorimetric kits containing Amplite™ IR can detect as low as 1 mU/mL of HRP and its signal can be measured with a fluorescence microplate reader at a max absorption at 640 nm and a max emission at 670 nm. Kits containing Amplite™ Red HRP substrate can detect as low as 10 µU/mL of HRP and its signal can be measured with a fluorescence microplate reader at a max absorption at 540 and a max emission at 590 nm. Absorbance microplate readers may also measure signals from both substrates, Amplite™ IR at ~647 nm and Amplite™ Red at ~576 nm. Both assays can be performed in a 96-well or 384-well microtiter plate and can easily be adapted to automation without a separation step.

Amplite™ Fluorimetric Goat Anti-Mouse and Goat Anti-Rabbit IgG-HRP Conjugate ELISA assay kits contain all the essential components, including our fluorogenic Amplite™ Red HRP substrate necessary for ELISA detection. Both kits provide an optimized and robust assay protocol. Amplite™ Fluorimetric Goat Anti-Mouse IgG-HRP Conjugate ELISA assay kit is capable of detecting as little as 0.4 ng/well of a monoclonal antibody. Amplite™ Fluorimetric Goat Anti-Rabbit IgG-HRP Conjugate ELISA assay kit is capable of detecting as little as 3 ng/well of a polyclonal antibody. Both signals can be measured with a fluorescent microplate reader at a max absorption at 540 and a max emission at 590 nm, or an absorbance microplate reader at ~576 nm. Each kit can easily be adapted to automation without a separation step and can be used for the assays in which goat anti-mouse or goat anti-rabbit IgG serve as the secondary detection agent.

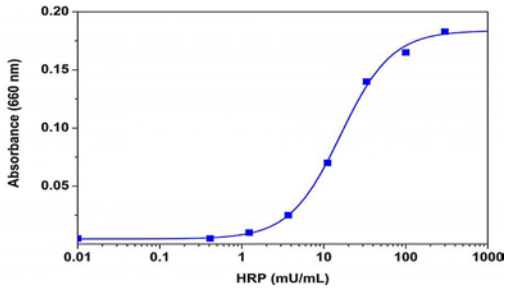

Figure 3.7 HRP dose responses were measured with Amplite™ Colorimetric Peroxidase Assay Kit (Cat# 11551) in a 96-well white wall/clear bottom plate. As low as 3 mU/mL of peroxidase was detected.

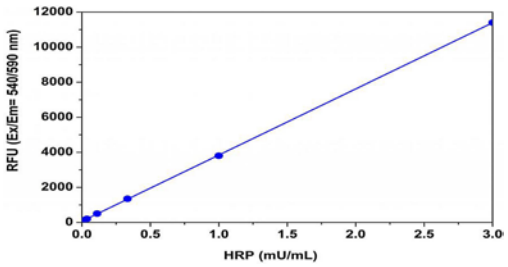

Figure 3.8 HRP dose responses were measured with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11552) in a 384-well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 10 µU/mL of peroxidase was detected with 30 minutes incubation (n=3).

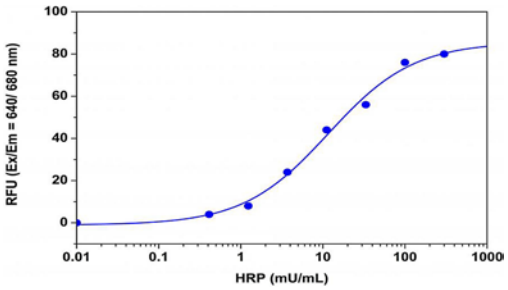

Figure 3.9 HRP dose responses were measured with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11553) in a 384-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 1 mU/mL of peroxidase was detected with 30 minutes incubation (n=3).

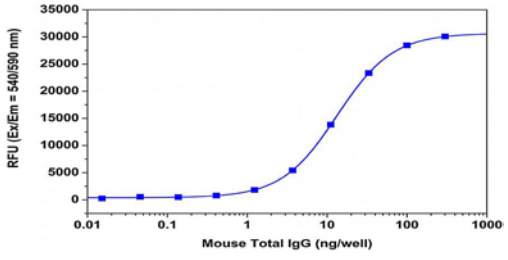

Figure 3.10 Detection of total mouse IgG using Amplite™ Fluorimetric Goat Anti-Mouse IgG-HRP Conjugate ELISA Kit (Cat# 11540). Mouse IgG was diluted into 3 µg/mL and made 1 to 3 serial dilutions in 0.2 M sodium bicarbonate buffer, pH 9.4. 100 µL/well serial dilutions were coated into a 96-well solid black plate at 4 oC overnight, and blocked with 3% milk in PBS and 0.02% Tween-20 at 4 oC overnight. The wells were washed, and assayed using the reagents. 1 to 5000 dilutions of goat anti-mouse IgG, HRP conjugate were used. The reactions were incubated for 10 to 60 minutes and then measured for fluorescence at Ex/Em = 540/590 nm using Gemini fluorescence micro-plate reader. As low as 0.4 ng/well of total mouse IgG was detected with 10 minutes incubation (n=3).

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Buccutite™ Conjugation Principle

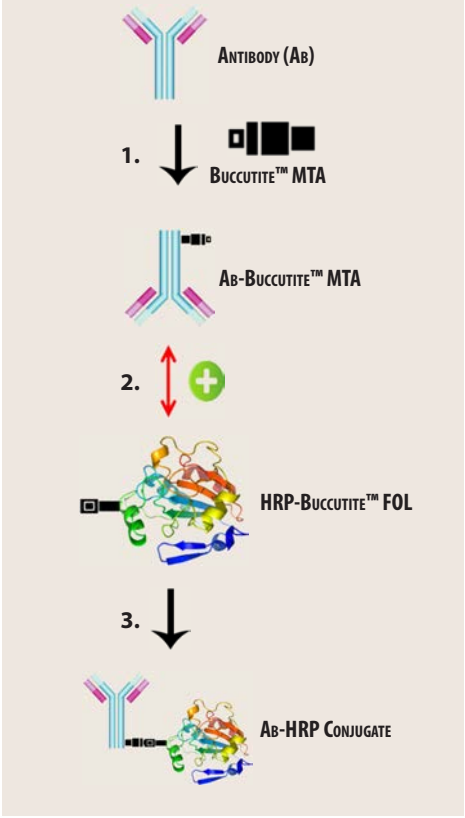

Figure 3.11 Conjugation process for ReadiLink Peroxidase (HRP) Antiobdy Conjugation Kit (Cat# 5503):

1. Desired antibody is activated with MTA.
2. Activated IgG-Buccutite™ MTA and preactivated HRP-Buccutite™ FOL are mixed.
3. HRP-labeled antibody is ready to use for desired immunoassay application.

Buccutite™ HRP Conjugation Technology

Our ReadiLink™ Peroxidase (HRP) Antibody Conjugation Kit was developed using our outstanding Buccutite™ protein crosslinking technology. The conjugation kit was designed for preparing horseradish peroxidase (HRP) conjugates directly with proteins, peptides, and other ligands that contain a free amino group. The HRP provided in our kit has been pre-activated with our proprietary linker Buccutite™ FOL, which can be directly used for conjugation. The Buccutite™ FOL -activated HRP readily reacts with Buccutite™ MTA-containing molecules under extremely mild neutral conditions without requiring a catalyst. Compared to commonly used SMCC and other similar technologies, our Buccutite™ bioconjugation system is much more robust and easier to use. It enables faster and quantitative conjugation of biomolecules with higher efficiencies and yields.

Besides the above-described ReadiLink™ HRP conjugation kit, we also offer the only commercially available ReadiUse™ preactivated HRP NHS ester. It is the mono NHS ester of HRP which can be used to readily label proteins such as antibodies or other biologicals molecules that have an amino group. ReadiUse™ preactivated HRP NHS esters are robust and easy to use, labeling antibodies with a simple mixing step. For example, the basic solution of an antibody (pH 8.5-9.0) can be directly mixed with the HRP NHS ester, and then shaken for 1-2 hours. In most cases, the resulted solution can be directly used for ELISA assays without further purification. Our ReadiUse™ preactivated HRP NHS ester, provides the most robust method for the conjugation of antibodies with HRP. Any suitable amount of antibody, less than 1 mg/mL, can be readily conjugated to the preactivated HRP NHS ester without further purification.

Detection of Alkaline Phosphatase and Its Conjugates

Cells utilize a wide variety of phosphate and polyphosphate esters as enzyme substrates, second messengers, membrane structural components and vital energy reservoirs. Alkaline and acid phosphatase are commonly used enzymes which hydrolyze phosphate monoesters to an alcohol and inorganic phosphate. Conjugates of calf intestinal alkaline phosphatase are extensively used as secondary detection reagents in ELISA, immunohistochemistry techniques, and Southern, Northern and Western blot analyses. For example, phosphatases serve as enzyme markers, allowing researchers to identify primordial germ cells to distinguish subpopulations of bone marrow stromal cells and to investigate in vitro differentiation in carcinoma cell lines. PALP-1, the gene for human placental alkaline phosphatase, has been used as eukaryotic reporter gene that is superior to lacZ for lineage studies in murine retina. The gene has also been engineered to produce a secreted alkaline phosphatase, allowing quantitation of gene expression without disrupting the cell.

Alkaline phosphatase (ALP) (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing phosphate groups is called dephosphorylation. An important use of alkaline phosphatase is as a label for enzyme immunoassays.

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Alkaline phosphatase is a highly sensitive enzyme for ELISA, immunohistochemistry, and Northern, Southern and Western blot applications. It is widely used in various biological assays and ELISA-based diagnostics.

Colorimetric Detection of ALP and Its Conjugates

Amplite™ Colorimetric Alkaline Phosphatase Assay Kit uses pNPP, a chromogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts, and on solid surfaces such as PVDF membranes. The kit provides an optimized "mix and read" assay protocol which is compatible with HTS liquid handling instruments. Its signal can be easily read by an absorbance microplate reader at ~400 nm. This Amplite™ Colorimetric Alkaline Phosphatase Assay Kit can be performed in a convenient 96-well or 384-well microtiter-plate format and can easily be adapted to automation without a separation step.

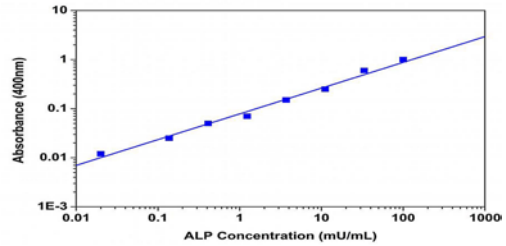

Figure 3.12 Alkaline phosphatase dose responses were measured with Amplite™ Colorimetric Alkaline Phosphatase Assay Kit (Cat# 11950) in a white/clear bottom 96-well plate using a NOVOstar microplate reader (BMG Labtech). As low as 0.3 mU/mL of alkaline phosphatase can be detected with 30 minutes incubation time (n=3).

Fluorimetric Detection of ALP and Its Conjugates

Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit, Cat# 11952, uses our MUP Plus™-based coumarin substrate. This fluorogenic phosphatase substrate, is excellent at quantifying alkaline phosphatase activity in solutions and in cell extracts. Similar to MUP, MUP Plus™ is sensitive to phosphatase-induced hydrolysis, which gives the halogenated coumarin substrate its intense blue fluorescence. Furthermore, the substantially lower pKa of the coumarin fluorophore makes this MUP Plus™ assay much less pH-dependent. MUP Plus™ substrates share nearly identical spectral properties with MUP, making it readily compatible with many fluorescence-based systems equipped with MUP settings. It can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a fluorescence microplate reader at a max absorption at ~360 nm and a max emission at ~450 nm.

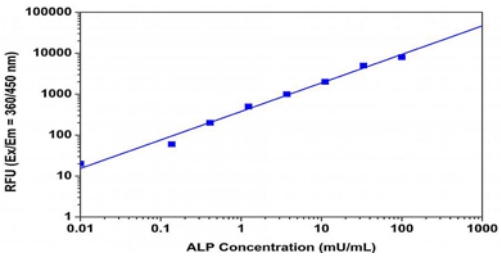

Figure 3.13 Alkaline phosphatase dose responses were measured with Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit (Cat# 11952)in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.1 mU/mL of alkaline phosphatase can be detected with 30 minutes incubation time (n=3).

Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit, Cat# 11953, uses fluorescein diphosphate (FDP), which is a highly sensitive green fluorogenic phosphatase substrate, to quantify the alkaline phosphatase activity in solutions and in cell extracts. FDP is an excellent substrate for alkaline phosphatase in ELISA assays, providing detection limits at least 50 times lower than those obtained by the chromogenic substrate pNPP. It can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a fluorescence microplate reader at a max absorption at ~490 nm and a max emission at ~525 nm.

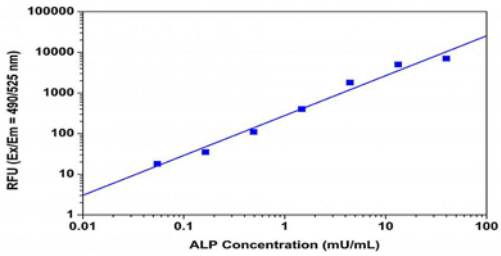

Figure 3.14 Alkaline phosphatase dose responses were measured with Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit (Cat# 11953) in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.1 mU/mL of alkaline phosphatase can be detected with 30 minutes incubation time (n=3).

Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit, Cat# 11954, uses a SunRed™-based substrate. This weakly fluorescent SunRed™-based substarte is sensitive to phosphatase-induced hydrolysis, which gives the SunRed™ fluorophore an intense red fluorescence. Upon phosphatase-induced hydrolysis, the SunRed™ phosphate solution has its absorption blue-shifted more than 100 nm. The maximum absorption of SunRed™ fluorophore at 633 nm makes this substrate an ideal NIR probe that can be readily detected with many fluorescence instrument systems often equipped with Cy5® settings.

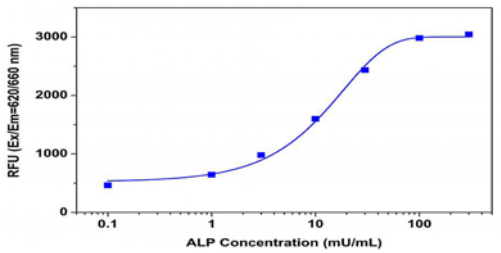

Figure 3.15 Alkaline phosphatase dose responses were measured with Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit (Cat# 11954) in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.3 mU/mL of alkaline phosphatase was detected with 60 minutes incubation (n=3).

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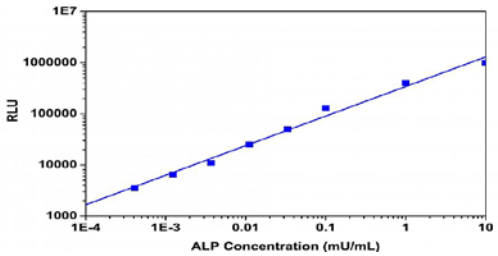

Figure 3.16 Alkaline phosphatase dose responses were measured with Amplite™ Luminometric Alkaline Phosphatase Assay Kit (Cat# 11956) in a 96-well white plate using a NOVOstar microplate reader (BMG Labtech). As low as 0.001 mU/mL of alkaline phosphatase was detected with 20 minutes incubation (n=3).

Based on the near infrared fluorescence of the SunRed™ fluorophore, the signal can be easily read by a fluorescence microplate reader at a maximum absorption at ~630 nm and a maximum emission at ~660 nm. This kit has been used for the high throughput screening of protein phosphatase inhibitors due to its low interference from the biological sample. It can be performed in a convenient 96-well or 384-well microtiter-plate format.

Amplite™ Luminometric Alkaline Phosphatase Assay Kit, Cat# 11956, uses D-luciferin phosphate as its luminogenic phosphatase substrate to quantify alkaline phosphatase activity in solution and in cells. D-luciferin phosphate is not recognized by luciferase until its phosphate group is removed resulting in luciferin. This kit provides and optimized "mix and read" assay protocol which is compatible with HTS liquid-handling instruments. Amplite™ Luminometric Alkaline Phosphatase Assay Kit can be readily performed in a 96-well or 384-well microtiter-plate format. Its signal can be easily read by luminescence microplate readers. The high sensitivity makes this kit ideal for immunoassays that require a low detection limit.

Product Ordering Information For HRP and ALP Substrates, Colorimetric, Fluorimetric and Luminescent Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
11000	Amplite™ ADHP	25 mg	571	585
11005	Amplite™ Blue	25 mg	324	409
11950	Amplite™ Colorimetric Alkaline Phosphatase Assay Kit *Yellow Color*	1 kit	405	None
11551	Amplite™ Colorimetric Peroxidase (HRP) Assay Kit *Blue Color*	1 kit	664	N/A
11952	Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Blue Fluorescence*	1 kit	360	449
11953	Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Green Fluorescence*	1 kit	490	514
11954	Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Near Infrared Fluorescence*	1 kit	646	660
11540	Amplite™ Fluorimetric Goat Anti-Mouse IgG-HRP Conjugate ELISA Assay Kit *Red Fluorescence*	1 kit	571	585
11541	Amplite™ Fluorimetric Goat Anti-Rabbit IgG-HRP Conjugate ELISA Assay Kit *Red Fluorescence*	1 kit	571	585
11553	Amplite™ Fluorimetric Peroxidase (HRP) Assay Kit *Near Infrared Fluorescence*	1 kit	647	670
11552	Amplite™ Fluorimetric Peroxidase (HRP) Assay Kit *Red Fluorescence*	1 kit	575	590
11009	Amplite™ IR	1 mg	647	670
11956	Amplite™ Luminometric Alkaline Phosphatase Assay Kit *Luminescence*	1 kit	N/A	560
11559	Amplite™ Luminometric Peroxidase (HRP) Assay Kit	1 kit	425	N/A
11011	Amplite™ Red	1000 assays	571	585
11628	CF-MUP, sodium salt *Superior alternative to MUP*	10 mg	360	450
12512	D-Luciferin phosphate *CAS 145613-12-3*	1 mg	328	533
11600	FDP [Fluorescein diphosphate, tetraammonium salt] *CAS 217305-49-2*	5 mg	490	514
11050	Luminol [3-Aminophthalhydrazide]	1 g	355	411
11610	MUP, disodium salt [4-Methylumbelliferyl phosphate, disodium salt] *CAS 22919-26-2*	25 mg	360	449
11630	PhosLite™ Green	1 mg	345	520
11619	pNPP [4-Nitrophenyl phosphate, disodium salt] *CAS 4264-83-9*	25 mg	399	N/A
11001	ReadiUse™ ABTS Substrate Solution	1 L	420	N/A
11003	ReadiUse™ TMB Substrate Solution	1 L	650	N/A
11012	ReadiUse™ TMB Substrate Solution	100 mL	650	N/A
11010	Signal Guard™ HRP conjugate stabilizer	50 mL	N/A	N/A
11629	SunRed™ Phosphate	5 mg	646	659

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Antibody Development Tools and Reagents

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Features of cBSA and cOVA:

- More free amine groups than corresponding native proteins
- Net positive charge for strong binding to antigen presentation cells
- Stronger immune response compared to native proteins

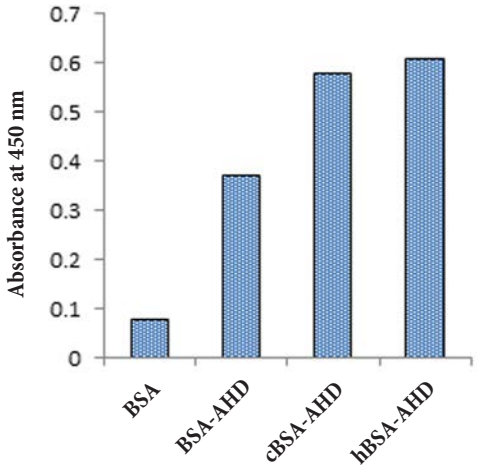

Figure 4.1 Rabbits are immunized with BSA, BSA-AHD (Aminohydantoin), cBSA-AHD and hBSA-AHD conjugates. Antisera from immunized rabbits are tested for the immune response against AHD. Data indicate cBSA-AHD and hBSA-AHD exhibit a stronger immune response to AHD in comparison with BSA-AHD.

Features of Protein A/G Agarose Resin:

- Recombinant proteins with a high purity
- Retain antibody activity and orient for maximum binding to Fc domain
- Lack of non-specific albumin binding
- Inert and stable

Carrier Proteins

Many proteins are suitable as carrier proteins used to couple peptides and haptens via thiol, amine or carboxyl groups, to generate antibodies against almost any molecules. Keyhole Limpet Hemocyanin (KLH) ($4.5x10^5$ to $1.3x10^7$ kDa), from genetically distinct mollusk is commonly used in the preparation of effective immunogen conjugated with small molecules.

Bovine Serum Albumin (BSA), which is a smaller protein than KLH at 67 kDa, contains 59 lysine residues, of which 30-35 are available for coupling. When BSA is cationized by the replacement of anionic carboxyl groups with cationic aminoethylamide groups, the modified forms of BSA, cBSA by ethylenediamine, add more free amine groups for conjugation. Additionally, conjugation with small molecules also elicits a stronger immune response to small haptens in comparison with the native BSA even in the absence of adjuvants (Figure 4.1).

Ovalbumin (OVA) is a protein containing 20 lysine residues, most of which are available for conjugation. While ovalbumin is less immnunogenic than KLH and BSA, it is more soluble in DMSO, which is important for conjugation with some organic haptens.

All the carrier proteins mentioned above can be coupled to peptides and haptens via the commonly used EDC or NHS ester crosslinkers. AAT Bioquest offers ReadiLink™ BSA and KLH bioconjugation kits custom conjugations of small molecules, oligo and peptides.

Protein A/G-Agarose Conjugates

Protein A is a bacterial protein possessing a high binding affinity for the F_c portion of different classes and subclasses of immunoglobulins from a variety of species (Table 6). AAT Bioquest's protein A conjugates are prepared with the highly purified 43 kDa recombinant protein A that is free of staphylococcal enterotoxins. Immobilized Protein A is ideal for polyclonal IgG purification from human, rabbit, pig, dog or cat serum.

Protein G is also a recombinant protein with a molecular weight of 21 kD, which is free of albumin and F_{ab} binding domains found in the native protein G. Unlike protein A, protein G has a better binding affinity to some IgGs, such as human IgG3, mouse IgG1 and immunoglobulins from rat, sheep and horse (Table 6), which makes protein G conjugates particularly valuable. Immobilized Protein G is ideal for polyclonal IgG purification from mouse, human, bovine, goat, and sheep serum, including human IgG3 and mouse IgG1 isotypes.

Secondary Antibody-Agarose Conjugates

Agarose antibody conjugates are meticulously prepared with either goat anti-mouse or goat anti-rabbit (H&L) IgGs at a binding capacity ideal for the affinity purification of the target IgG, immunoprecipitation and removal of cross-reactive antibodies. Anti-

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IgG Secondary Antibody Agarose can be used for immunoaffinity chromatography of polyclonal or monoclonal mouse IgG antibodies. Once bound, non-specific antibodies may be removed by copious washing followed by elution of the specific antibody at low pH.

Features of Anti-IgG Antibody Agarose Resins:

- High binding capacity: up to 2.0 mg of mouse/rabbit IgG, or 15 µg of biotin per milliliter of beads
- Stable conjugation of IgG or streptavidin on crosslinked agarose beads
- Excellent chemical and physical stability

Table 6. Binding affinity of Protein A and Protein G with various species

Species	Immunoglobulin	Protein A	Protein G	Protein A/G
Goat	IgG	+	++	++
Guinea pig	IgG	+	++	++
Horse	IgG	-	++	+++
Human	IgG1	++	++	++
	IgG2	++	++	++
	IgG3	-	++	++
	IgG4	++	++	++
	IgM	++	-	++
	IgA	++	-	++
	IgE	++	-	++
Mouse	IgG1	-	++	++
	IgG2a	++	++	++
	IgG2b	++	++	++
	IgG3	++	++	++
Pig	IgG	++	++	++
Rabbit	IgG	++	++	++
Rat	IgG	-	+	+
Sheep	IgG	-	++	++

++ Strong binding. + Moderate binding. – Weak or no binding.

Product Ordering Information For Antibody Development Tools and Reagents

Catalog	Description	Unit Size
5600	cBSA (Ethylenediamine-modified BSA)	10 mg
5605	cOvalbumin (ethylenediamine--modified OVA)	10 mg
55010	Goat anti-mouse IgG (H&L) Agarose	10 mg
55015	Goat anti-rabbit IgG (H&L) Agarose	10 mg
55000	Protein A-Agarose Resin	5 mL
55005	Protein G-Agarose Resin	1 mL

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Website: http://www.assaymatrix.com

Life Research Pty Ltd.
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Website: http://www.liferesearch.com

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Website: http://www.biomol.de

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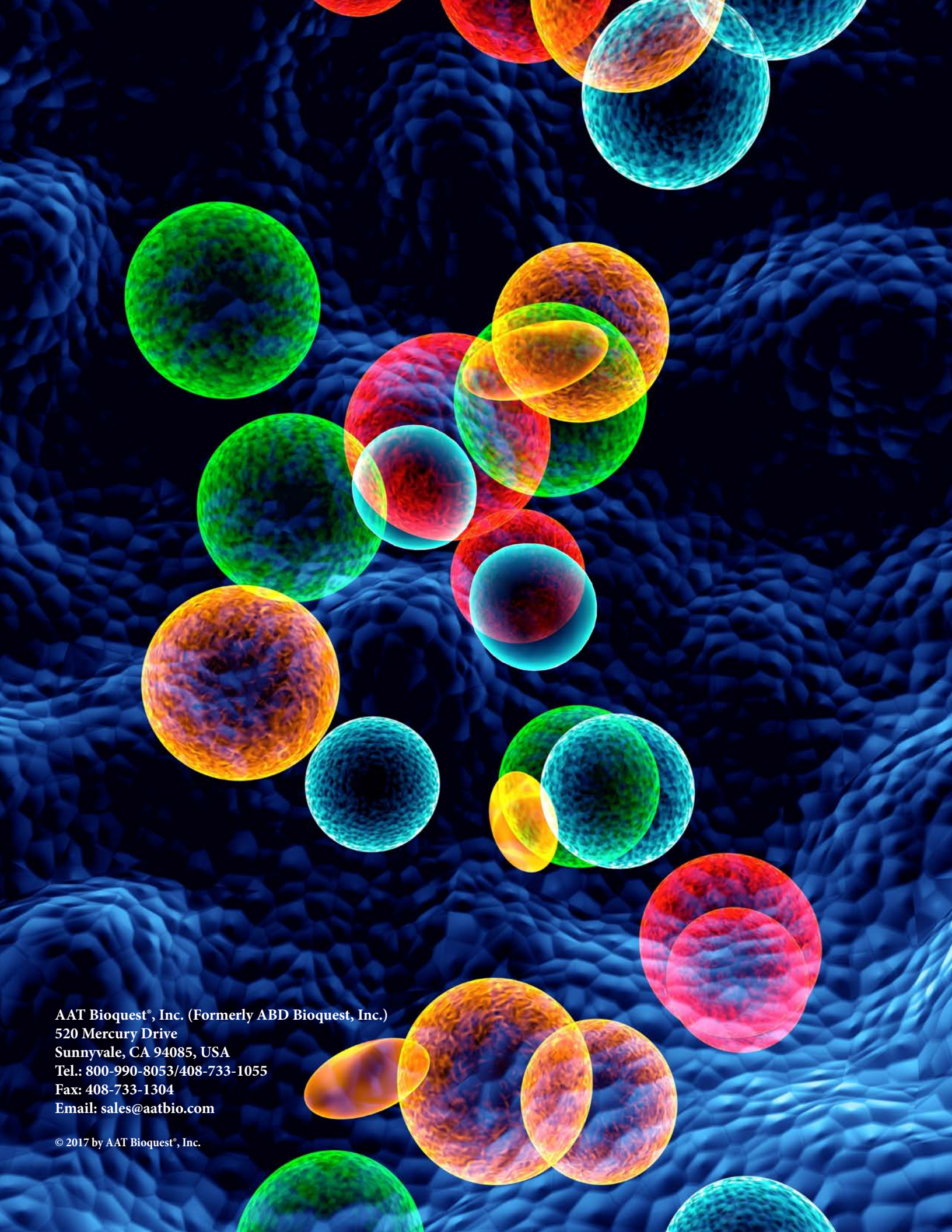

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