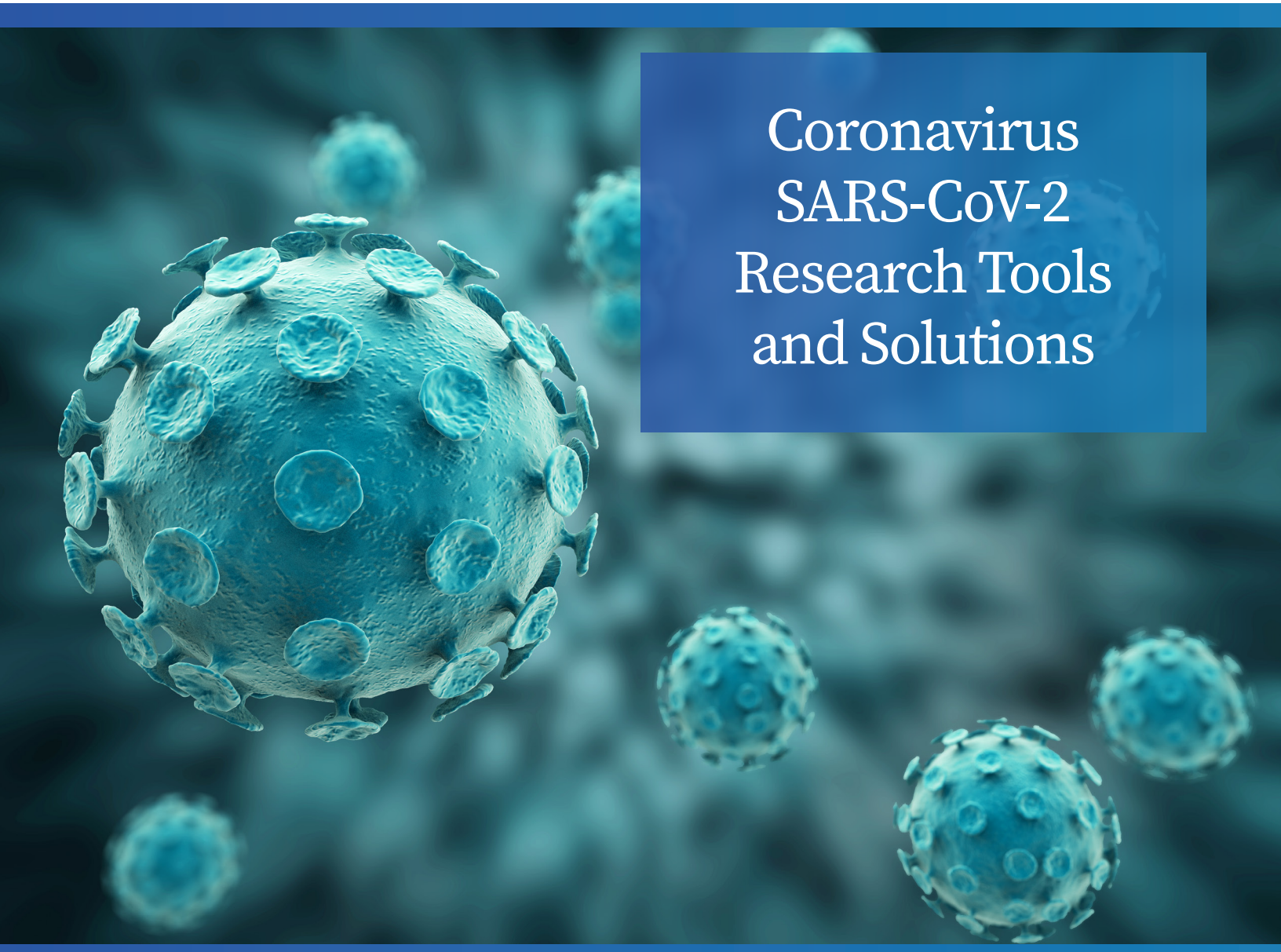
Vol. 9
Issue 2

ASSAYWISE LETTERS

LIFE SCIENCE RESOURCES AND APPLICATIONS

Fall 2020



Coronavirus SARS-CoV-2 Research Tools and Solutions

ALSO FEATURING

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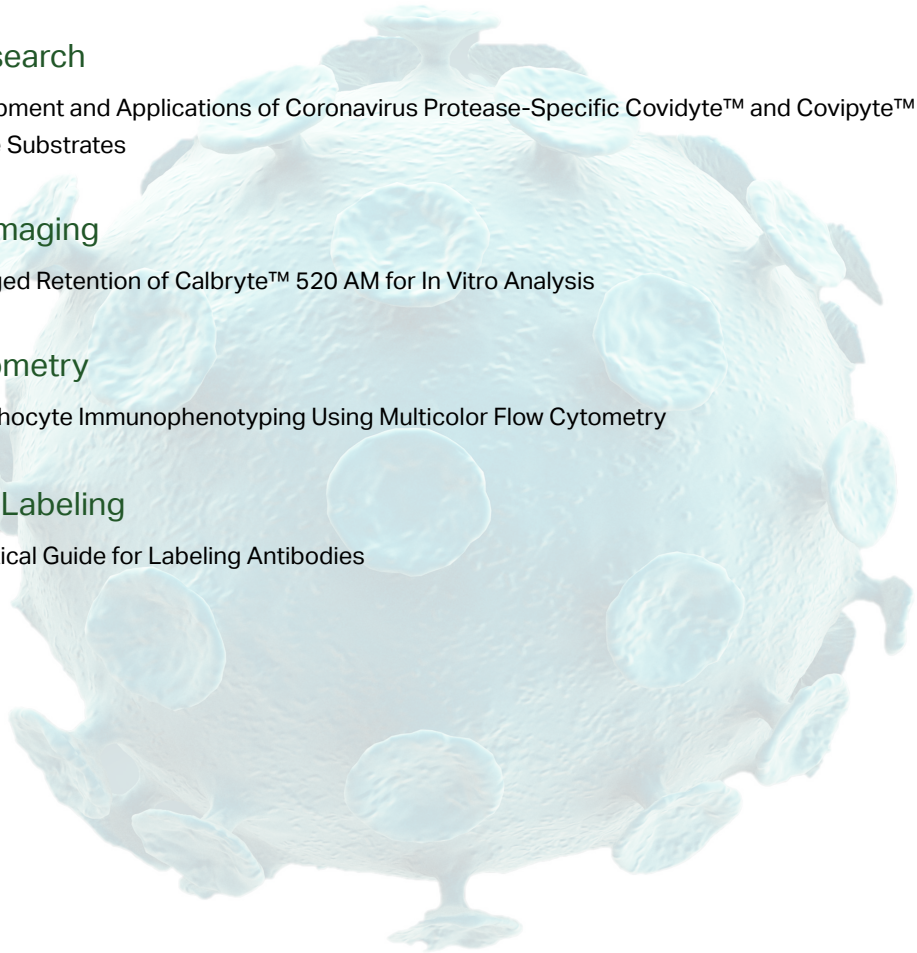


Development and Applications of

Coronavirus Protease-Specific Covidyte™ and Covipyte™ Peptide Substrates

Abstract

There is a high level of similarity in both amino acid sequence and structure between the SARS and SARS-CoV-2 virus, particularly in regards to the catalytic active sites of their proteases. Several coronavirus-specific amino acid sequences were discovered for use in investigating enzyme inhibitors for SARS, and have since been repurposed for SARS-CoV-2 research. By conjugating these sequences to FRET pairs, several fluorescent substrates have been developed to accurately measure protease activity. These substrates can be employed in sensitive assays for research into development of possible protease inhibitors for SARS-CoV-2 antiviral therapies.

Introduction

Detected in late 2019 and subsequently spreading across the globe, the pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represents the worst threat to human health in over a century.

Also referred to as Covid-19, the virus has marshaled unprecedented efforts from the global scientific community in research into possible treatments and vaccines. There are several avenues of inquiry being pursued, of which one of the most promising is inhibiting essential enzymatic catalysis that the virus requires to function and replicate itself. Identifying and targeting proteases were helpful in identifying successful therapies during previous health crises, such as HIV, as well as the viral predecessor of the current pandemic, the related SARS-CoV pathogen that caused an epidemic in 2003.[1]

SARS-CoV-2 has two cysteine proteases: the papain-like protease PLpro, and the chymotrypsin-like protease 3CLpro, (sometimes called the main protease or Mpro) both of which are high-priority therapeutic targets.[2]

Via prior research with related coronaviruses, work on mapping the sequences and structure of these has led to the development of coronavirus-specific substrates which can be used to detect and measure enzymatic activity. These allow accurate evaluation of treatments, and pave the way to further exploration into possible inhibitors and treatments.

Sequence and Structure Conservation Between Previous Coronaviruses and SARS-CoV-2

Of particular interest to medical researchers is the knowledge that the catalytic sites for proteases are very highly conserved. There is only one amino acid difference (ser46) at the protease active site between SARS-CoV-2 and SARS.[1] This conservation of sequence strongly indicates that observed behavior and responses of these sites can be applied with confidence across types.

Although the viruses aren't identical, comparative experiments demonstrate behavioral correlation. Unfortunately, the variations in viral structure that do exist lead to differences in binding affinities and presumed efficacy of experimental inhibiting compounds.

Given the similarities, however, the research done during and in the wake of the SARS epidemic is able to be repurposed

into promising investigations for the closely-related SARS-CoV-2 pandemic.

As seen in Figure 1, the overall structures of coronaviruses such as SARS, MERS, and SARS-CoV-2 overlap extensively. The SARS and SARS-CoV-2 genome sequences in particular have an 89.1% similarity.[3] This is significant for researchers, since the structural similarity implies that similar treatments might be effective. The sequence overlap also means that there are many identical or near-identical stretches of amino acid sequences, including the functional sites for many enzymatic targets including both proteases. This permits prior knowledge of the functional sites for SARS to be immediately applicable to investigations into inhibiting the enzymatic targets of SARS-CoV-2.

Coronavirus 3CLpro Protease as a Treatment Target

The PLpro and 3CLpro proteases are both essential for the function of SARS-CoV-2.

PLpro has several roles in signaling and other processes, including replication, but 3CLpro is central to viral replication, making it the primary target of anti-infection research. 3CLpro is responsible for producing functional proteins during viral replication via hydrolysis of 2 larger polyproteins (pp1a and pp1ab).4 Without this enzyme, replication of the virus would be impossible. Additionally, the 11 known cleavage sites for this enzyme are unique and are not shared with human proteases, minimizing the risk of treatment toxicity.

The 3CLpro protease is unique among the many possible therapeutic targets of SARS-CoV-2 in that the vast amounts of research done on the prior SARS coronavirus provides a generous foundation for research into treatments. Given that finding possible therapies aside from current symptomatic treatments is of a time-sensitive nature, having that background is promising. Some compounds used during the SARS epidemic are already seeing experimental clinical use during the current pandemic.[3] As research continues worldwide, other potential drugs from these prior findings are likely to be improved or discovered that work as well or better against SARS-CoV-2.

Covidyte™ & Covipyte™ Fluorogenic Substrates: Research Applications

A fundamental part of laboratory science is the development of the materials and methodology to accurately observe and measure a given process or compound. To measure the protease activity of SARS-CoV-2, several techniques have been created, with more constantly being proposed.

The most sensitive and efficient method of measuring the SARS-CoV-2 enzyme activity uses a biochemical phenomenon

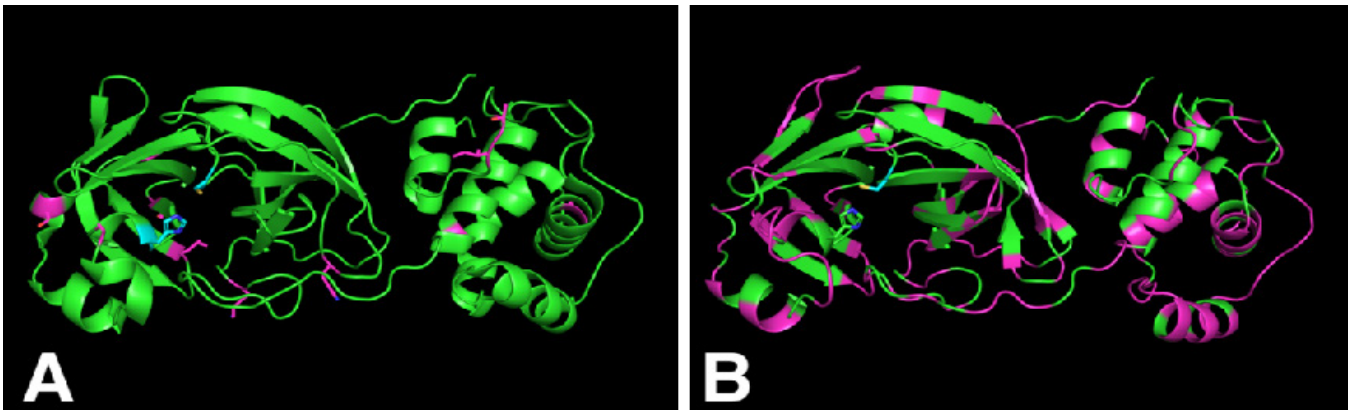


Figure 1. X-Ray Crystal Structure Comparison of bat HKU4, SARS, & SARS-CoV-2 Proteases. Crystal structures from the Protein Data Bank (www.rcsb.org). Section A (PDB:2z9j) represents SARS, with green ribbons showing major homology. Blue sections represent the catalytic residues, and pink portions represent differences in SARS-CoV-2. Section B (PDB:2ynb) represents bat coronavirus HKU4 protease, with the same color representations. There is extensive overlap between coronavirus proteases, with SARS and SARS-CoV-2 being largely identical. Image from Homology Models of Coronavirus 2019-nCoV 3CLpro Protease.[2]

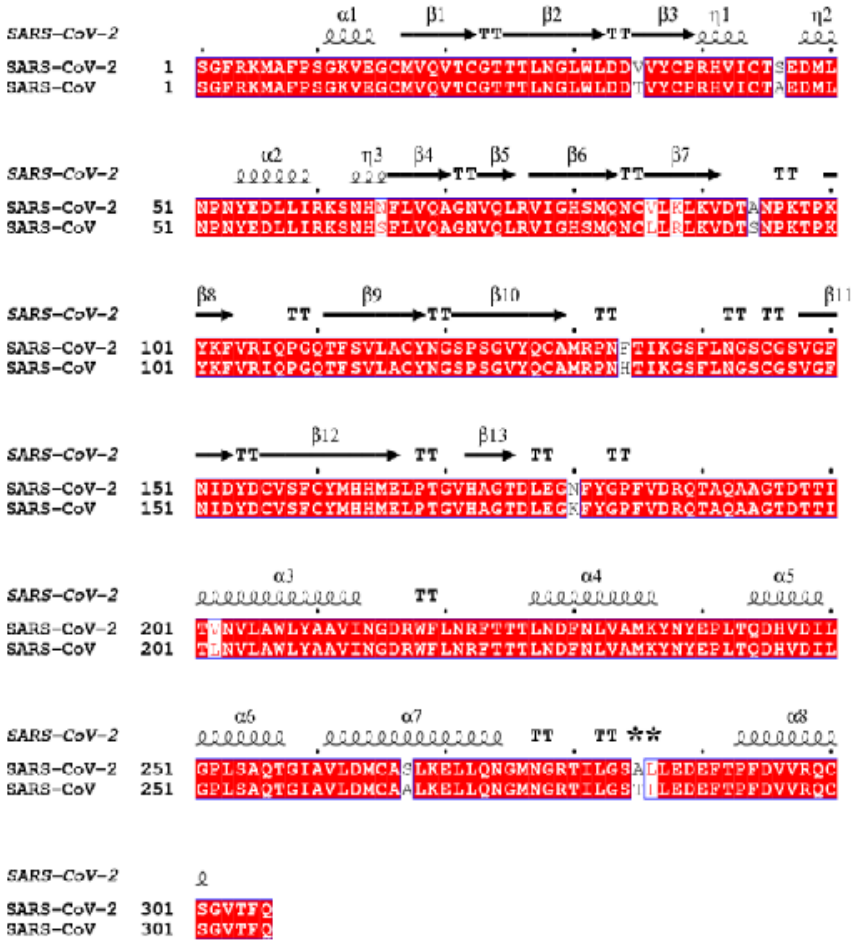


Figure 2. Comparison of the Amino Acid Sequences of Coronavirus 3CLpro Protease between SARS-CoV-2 and SARS. Identical amino acid chains are highlighted in red. Notable features of the crystal structure of 3CLpro are indicated above the relevant sequences. α = alpha-helix, β = b-strand, η = 310-helix, TT = turn. Image from Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved ?-ketoamide inhibitors.

known as Förster resonance energy transfer (FRET). In very simple terms, FRET is the energy exchange from a high-energy excited 'donor' molecule to an 'acceptor' molecule over a distance smaller than 10 nm.

Within that distance, the excited donor molecule transfers energy to the acceptor via dipole-dipole interaction. When this energy transfer occurs between two fluorophores, the overall fluorescence intensity is quenched. There are many possible FRET pairs of donor/acceptors currently used in laboratories, with one of the most popular being EDANS/DABCYL. A variation on this energy exchange is known as 'dark' FRET, which is very similar, but instead of two fluorophores, consists of a pairing of an energy donor fluorophore and a 'dark' quencher chromophore. This is the interaction used for the Covidyte™ and Covipyte™ dark FRET substrates.

FRET is the basis for fluorescence-based activity assays using several amino acid peptides isolated for their efficacy in SARS enzymatic activity. For the investigation of 3CLpro protease, the first of these peptides is 12 amino acids in length, with the sequence VNSTLQSGLRKM, and the second is 14, with a sequence of KTSAVLQSGFRKME.6 Both of these sequences are specifically targeted by coronavirus proteases and not by human. By conjoining these amino acid sequences to FRET pairs, researchers can use the observed fluorescence as a sensitive measurement of enzymatic activity. When the protease cleaves the amino acid sequences, the conjoined fluorophore

pair breaks and no longer is quenched by FRET. The more protease activity is present, the higher the increase in observed fluorescence. Using both classic FRET pairs and improved modern versions, several fluorescent peptide substrates are available for research use.

For the investigation of PLpro protease, the longest of these peptides is 9 amino acids long, with a sequence of RELNGGAPI. There are also 3 smaller tetra-peptide substrates that have been developed, each only 4 amino acids in length, with sequences of: KAGG, KKAG, and LRGG.

The development of fluorescent substrates such as these allow not only improved measurement of protease activity levels, but more sensitive detection of these enzymes overall. Protease activity assays are often used to assess the efficacy of potential enzyme inhibitors or of general viral activity. By using FRET pairs in particular, the assays can employ a fluorescent microplate format or similar, permitting conversion to a high-throughput workflow.

Discussion

By using prior research on coronaviruses, and leveraging the structural similarities of SARS-CoV-2, the development of fluorescent substrates like Covidyte™ and Covipyte™ to investigate the behavior of both PLpro and 3CLpro coronavirus proteases is a promising tool for investigating possible antiviral treatments.

Using modern computational power to help model, hundreds of thousands of compounds can be assessed for inhibitory interactions, and the best-performing simulations selected for further experiments.

The continued combined efforts of researchers around the globe, and most particularly the highest levels of scientific cooperation ever seen, will be essential for preserving as many human lives as possible during the SARS-CoV-2 pandemic.

References

1. Chen, S., Chen, L. L., Luo, H. B., Sun, T., Chen, J., Ye, F., Cai, J. H., Shen, J. K., Shen, X., & Jiang, H. L. (2005). Enzymatic activity characterization of SARS coronavirus 3C-like protease by fluorescence resonance energy transfer technique. Acta pharmacologica Sinica, 26(1), 99–106. https://doi.org/10.1111/j.1745-7254.2005.00010.x
2. Fischer, A., Sellner, M., Neranjan, S., Smieško, M., & Lill, M. A. (2020). Potential Inhibitors for Novel Coronavirus Protease Identified by Virtual Screening of 606 Million Compounds. International Journal of Molecular Sciences, 21(10), 3626. https://doi.org/10.3390/ijms21103626
3. He, J., Hu, L., Huang, X., Wang, C. Zhang, Z., Wang, Y., Zhang, D., & Ye, W. (2020). Potential of coronavirus 3C-like protease inhibitors for the development of new anti-SARS-CoV-2 drugs: Insights from structures of protease and inhibitors. International Journal of Antimicrobial Agents, 56(2), 106055. https://doi.org/10.1016/j.ijantimicag.2020.106055
4. Kuo, C. J., Chi, Y. H., Hsu, J. T., & Liang, P. H. (2004). Characterization of SARS main protease and inhibitor assay using a fluorogenic substrate. Biochemical and biophysical research communications, 318(4), 862–867. https://doi.org/10.1016/j.bbrc.2004.04.098
5. Stoermer, Martin (2020): Homology Models of Coronavirus 2019-nCoV 3CLpro Protease. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.11637294.v3
6. Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., & Hilgenfeld, R. (2020). Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved ?-ketoamide inhibitors. Science (New York, N.Y.), 368(6489), 409–412. https://doi.org/10.1126/science.abb3405

Table 1. Covidyte™ and Covipyte™ substrates for screening coronavirus protease inhibitors.

Product	Unit Size	Cat No.
Covidyte™ ED450	100 Tests	13537
Covidyte™ ED450	1000 Tests	13538
Covidyte™ EN450	100 Tests	13535
Covidyte™ EN450	1000 Tests	13536
Covidyte™ IF670	100 Tests	13542
Covidyte™ IF670	1000 Tests	13543
Covidyte™ TF670	100 Tests	13540
Covidyte™ TF670	1000 Tests	13541
Covipyte™ EN450	100 Tests	13545
Covipyte™ EN450	1000 Tests	13546
Z-KAGG-AMC	1 mg	13552
Z-KKAG-AMC	1 mg	13554
Z-LRGG-AMC	1 mg	13550

Prolonged Retention of Calbryte™ 520 AM

for In Vitro Analysis

Abstract

Calcium-imaging is a sensitive method for monitoring the spatiotemporal dynamics of calcium signaling. Since intracellular concentrations are correlated with physiological and pathophysiological characteristics of the central nervous system (CNS), calcium imaging with fluorescent indicators provides an ultra-sensitive and accurate method for measuring calcium responses in vitro. In this technical note, we compared the performance of two fluorescent calcium indicators - Fluo-4 AM and Calbryte™ 520 AM - following prolong incubation periods post cell loading and measured the response upon ATP stimulation. Cells loaded with Calbryte™ 520 AM retained the dye for 24 hours post staining while exhibiting superior signal-to-noise characteristics. The longer retention of Calbryte™ 520 AM can be exploited to accomodate for the extended time required when monitoring calcium dynamics in acute neuronal tissue.

Introduction

Calcium is an important secondary messenger that plays a key role in different intracellular physiological processes, and due to its importance in regulating such processes, calcium imaging is widely used in many laboratories all over the world.[1] The source of calcium entry to the cytoplasm can be either external or internal, and its concentration is tightly maintained by calcium transporters that remove calcium ions rapidly to avoid cytotoxic effects. Cytosolic Ca+2 signals are generally classified as being either transient, sustained or oscillatory depending upon the degree and duration of calcium influx.[2] To satisfy these variations in calcium signaling, AAT Bioquest has pioneered major advancements in calcium detection by developing a series of indicators with wider wavelength options, brighter fluorescence intensities and better photostability enabling faster, more accurate measurements of calcium concentration and mobilization.

One limitation that has proved challenging in calcium imaging is the polar nature of calcium indicators. By their nature, negatively charged compounds are cell-impermeant and require either physically invasive loading techniques, such as microinjection, electroporation, or patch pipette, or chemical loading techniques to enter live cells. The latter, which was first introduced by Roger Tsien[5], offers a more convenient and passive method for loading hydrophilic dyes into live cells. In order to chemically load dyes, they must be first modified with acetoxymethyl (AM) esters. AM ester modification masks the negatively charged carboxylic groups and produces uncharged hydrophobic indicators, which can be passively loaded into live cells.

Following hydrolysis of the AM groups by intracellular proteases, indicators are released and stay inside the cells. However, the loading efficiency of calcium indicators varies between cell types due to organic anion transporters that can extrude the dye.

For that reason, probenecid, which is an organic anion transporter inhibitor, is used to improve dye retention by deactivating these pumps. Unfortunately, probenecid is not without caveats. Probenecid has been shown to adversely affect platelet aggregation and cytotoxicity, processes that involve intracellular Ca+2 homeostasis [6], and calcium flux kinetics of cyclic nucleotide gated ion channels resulting in reducing response of the agonists [7].

Here, we describe a novel indicator for calcium imaging applications, Calbryte™ 520. This indicator is part of the Calbryte™ series, a family of fluorescent dyes developed to monitor intracellular calcium. Calbryte™ 520 absorbs light at 493 nm and emits at 515 nm, making it spectrally similar to Fluo-4 (Ex/Em = 495/528 nm). However, Calbryte™ 520 is far superior than Fluo-4, particularly when comparing dye-retention and signal-to-noise ratios.

In the present study, Calbryte™ 520 AM and Fluo-4 AM were loaded into cells under the same conditions. ATP-induced calcium responses were measured using a fluorescence microscope after 0 hours, 4 hours and 24 hours of prolonged incubation. Comparisons were made for fluorescence intensity, signal-to-noise ratio, and dye retention. Additionally, Calbryte™ 520 was used to measure changes in calcium levels by flow cytometry after 0 hours, 24 hours and 48 hours of incubation.

Materials and Methods

Cell Culture

CHO-K1 cells were cultured at 37°C in a F12-K nutrient mixture containing heat inactivated serum (5% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin), under a humidified atmosphere containing 5% CO2.

HeLa cells were cultured at 37°C in DMEM containing heat inactivated serum (10% Fetal Bovine Serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin), under a humidified atmosphere containing 5% CO2. Jurkat cells were cultured at 37°C in RPMI containing heat inactivated serum (10% Fetal Bovine Serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin), under a humidified atmosphere containing 5% CO2.

Fluorescence microscopy Calbryte™ 520 AM and Fluo-4 AM

Cells were plated in 100 µL culture medium in a 96-well black wall/clear bottom costar plate (Greiner Bio-One, Kremsmunster, Austria) at 50,000 cells per well.

The next day, equal volumes of Fluo-4 AM (AAT Cat# 20550) and Calbryte™ 520 AM (AAT Cat# 20650)-loading solutions with final concentrations of 5 µM and 0.02% PF-127 (AAT Cat# 20053) in the absence of a probenecid were added to each well. Cells were incubated with the dye-loading buffer for 120 minutes

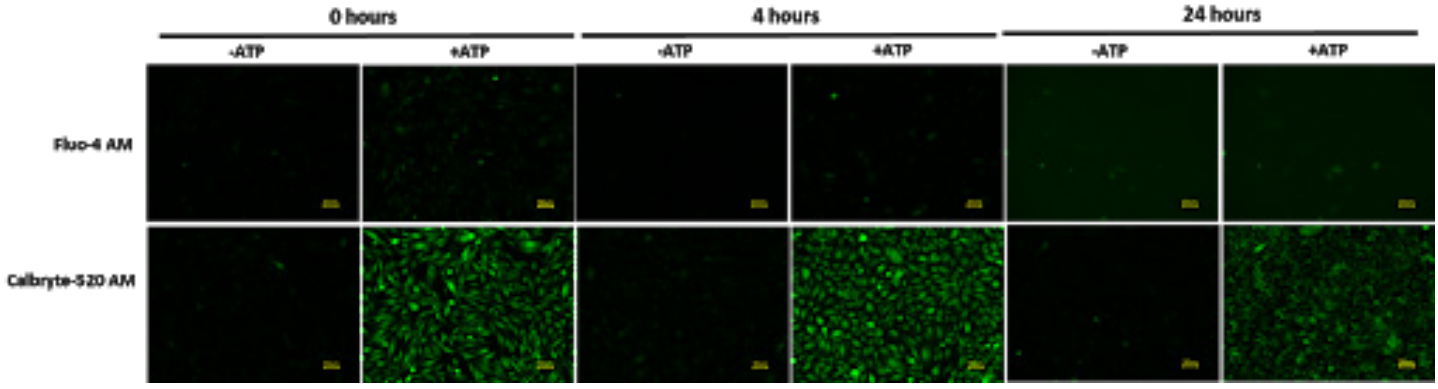


Figure 1. Response of endogenous P2Y receptor to ATP in CHO-K1 cells. CHO-K1 cells were stained with Fluo-4 AM or Calbryte™ 520 AM in HHBS without probenecid, and the cells were incubated at 37°C for 2 hours. After that, cells were grown in the cell culture medium. At indicated times, the cell culture medium was replaced with 200 µL HHBS and 50 µL of 50 µM ATP were added, and imaged with a fluorescence microscope (Keyence) using FITC channel.

at 37°C, 5% CO2 incubator. The dye loading solution was then removed, fresh cell culture medium (200 µL) was added to the plate and incubated at 37°C, 5% CO2 incubator.

Response was measured at the indicated times by adding 5X solution of ATP to stimulate P2Y1 signaling. Images were acquired with fluorescence microscopes (Keyence) using the FITC filter set.

Flow cytometry analysis for longer retention tests

Cells were plated in 10-cm flask at 50,000 cells per well. The next day, equal volumes of Calbryte™ 520 AM-loading solutions with final concentrations of 5 µM were added to each well and incubated for 60 minutes at 37°, 5% CO2 incubator.

The dye loading solution was then removed, fresh cell culture medium was added to the plate and incubated at 37°, 5% CO2 incubator. The cells were collected at indicated intervals and washed once with HH buffer. Flow cytometry was performed with FITC channel using NovoCyte® flow cytometer from ACEA Biosciences.

Results

Prolonged retention of Calbryte™ 520 AM

Flow cytometry analysis was performed to assess Calbryte™ 520 AM retention in cells following prolonged periods of incubation.

Jurkat cells were stained with Calbryte™ 520 AM, washed and then grown in cell culture medium for several days. After a period of 2 days, it was observed that Calbryte™ 520 AM was well-retained in cells (Figure 1), making it a suitable choice for long-term calcium tracing in vitro.

Calcium detection after a prolonged retention in cells

This study aimed to demonstrate the length of time cells, loaded with calcium indicators, can be maintained with the dye to produce reliable and reproducible data about cell function.

We loaded the HeLa (See supplemental figure 1) and CHO-K1 (Figure 2) cells with Fluo-4 AM and Calbryte™ 520 AM in the absence of probenecid and incubated for 120 minutes at 37°C, 5% CO2 incubator.

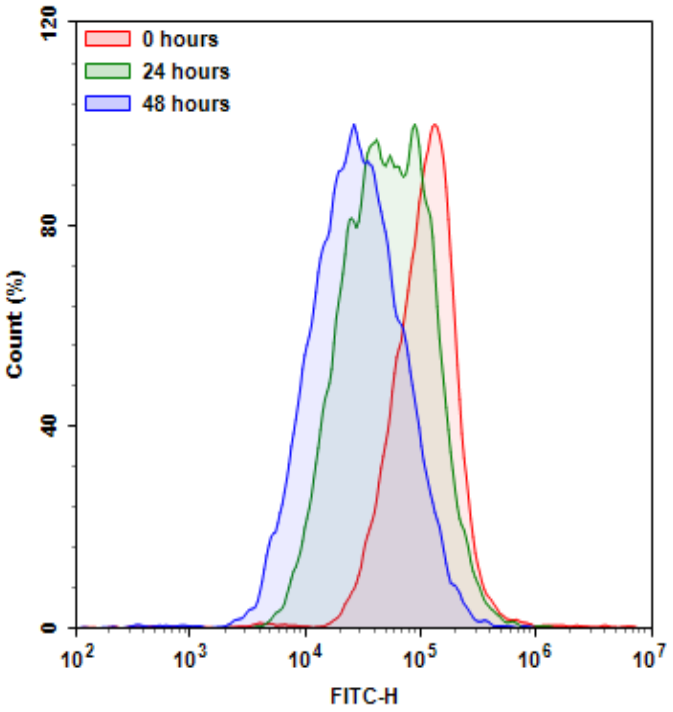


Figure 2. Retention test for Calbryte™ 520 AM in Jurkat cells using flow cytometry. Jurkat cells were stained with Calbryte™ 520 AM at 5 µM for 1 hour at 37°C incubator. Cells were washed once and grown in cell culture medium and subjected to flow cytometry analysis at indicated times. The response was recorded using the FITC channel in NovoCyte flow cytometer. Data shown are representative of at least 3 biological replicates.

After incubation, cells were shifted to full cell culture medium and stimulated P2Y signaling using ATP solution at indicated times.

We showed that adding ATP solution will stimulate the cells and induce fluorescence in Calbryte™ 520 AM containing cells post loading up to 24 hours but the same response was not observed in Fluo-4 AM containing samples possibly due to its poor retention property.

Conclusions

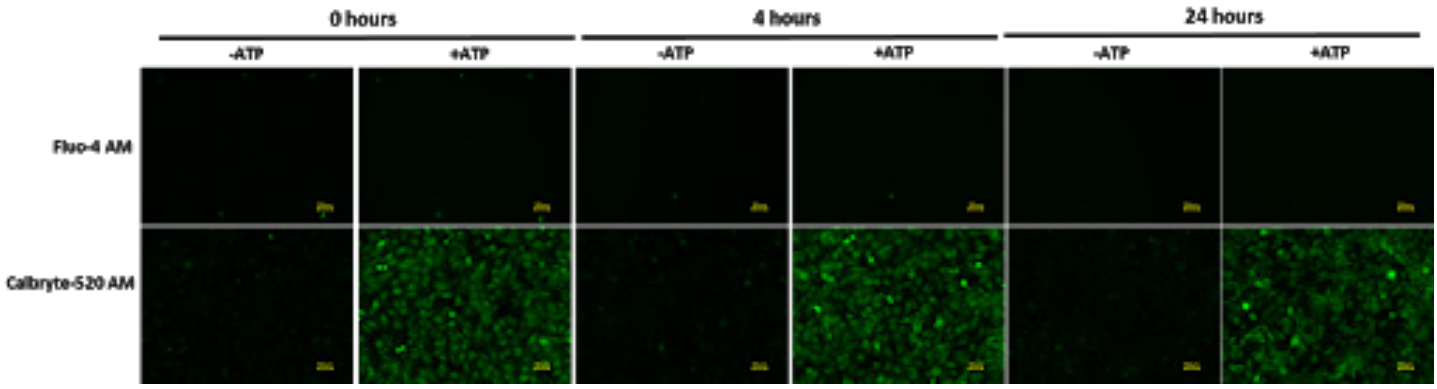
Our comparative study demonstrated the cells loaded with Calbryte™ 520 AM were capable of retaining the indicator for prolonged periods of time (over 48 hours) without the use of probenecid.

The longer duration of staining would immensely help staining the tissue samples which requires longer workaround time.

References

1. M.D. Bootman, K. Rietdorf, T. Collins, S. Walker, M. Sanderson, Ca2+-Sensitive Fluorescent Dyes and Intracellular Ca2+ Imaging, Cold Spring Harb. Protoc. 2013 (2013) pdb.top066050. https://doi.org/10.1101/pdb.top066050.
2. D.E. Clapham, Calcium Signaling, Cell. 131 (2007) 1047–1058. https://doi.org/10.1016/j.cell.2007.11.028.
3. S.-H. Chung, M.-J. Kim, J.-Y. Lee, J.-H. Chung, EFFECTS OF PROBENECID ON PLATELET AGGREGATION AND CYTOTOXICITY: DRAWBACKS OF THE USE OF PROBENECID IN MONITORING INTRACELLULAR CALCIUM METABOLISM, Thromb. Res. 85 (1997) 345–350. https://doi.org/10.1016/S0049-3848(97)00020-0.
4. C. Grienberger, A. Konnerth, Imaging Calcium in Neurons, Neuron. 73 (2012) 862–885. https://doi.org/10.1016/j.neuron.2012.02.011.
5. R.Y. Tsien, A non-disruptive technique for loading calcium buffers and indicators into cells, Nature. 290 (1981) 527–528. https://doi.org/10.1038/290527a0.
6. P. Uhlén, N. Fritz, Biochemistry of calcium oscillations, Recent Prog. Mol. Sci. Rev. Karolinska Institutet Its 200-Year Anniv. 396 (2010) 28–32. https://doi.org/10.1016/j.bbrc.2010.02.117.
7. A. Visegrády, A. Boros, Z. Némethy, B. Kiss, G.M. Keser?, Application of the BD ACTOneTM Technology for the High-Throughput Screening of Gs-Coupled Receptor Antagonists, J. Biomol. Screen. 12 (2007) 1068–1073. https://doi.org/10.1177/1087057107309035.

Supplemental Figures



Supplemental Figure 1. Response of endogenous P2Y receptor to ATP in HeLa cells. HeLa cells were stained with Fluo-4 AM or Calbryte™ 520 AM in HHBS without probenecid, and the cells were incubated at 37°C for 2 hours. After that, cells were grown in the cell culture medium. At indicated times, the cell culture medium was replaced with 200 µL HHBS and 50 µL of 50 µM ATP were added, and imaged with a fluorescence microscope (Keyence) using FITC channel.

Product	Unit Size	Cat No.
Calbryte™ 520 AM	2x50 µg	20650
Calbryte™ 520 AM	10x50 µg	20651
Calbryte™ 520 AM	1 mg	20653
Fluo-4 AM *Ultrapure Grade* *CAS 273221-67-3*	1 mg	20550
Fluo-4 AM *Ultrapure Grade* *CAS 273221-67-3*	10x50 µg	20551
Fluo-4 AM *Ultrapure Grade* *CAS 273221-67-3*	5x50 µg	20552
Pluronic® F-127 *Cell culture tested *	10 g	20050
Pluronic® F-127 *20% solution in DMSO*	10 mL	20052
Pluronic® F-127 *10% solution in water*	10 mL	20053
Probenecid *Cell culture tested, CAS 57-66-9*	10x72 mg	20060
ReadiUse™ probenecid *25 mM stabilized aqueous solution*	10x10 mL	20062

T Lymphocyte Immunophenotyping

Using Multicolor Flow Cytometry

Abstract

Cluster of differentiation (CD) antigens are a complex and highly organized collection of surface molecules expressed on cells of the immune system that play key roles in immune cell-cell communication, sensing the microenvironment and in adaptive immunity. During lymphocyte maturation, immune cells will express complex variations of CD antigens on their cell surface, some of which are lost, while others are acquired at various stages either by interactions with antigen presenting cells (APC) or with other cells of the immune system. In flow cytometric immunophenotyping, monoclonal antibodies targeting CD antigens facilitate the identification and characterization of leukocytes and lymphocyte sub-populations. By using different combinations of CD antibodies, it is possible to characterize the cell surface immunophenotypes of different leukocyte sub-populations, including the functionally distinct mature cell subpopulations of B cells, helper T cells (TH), cytotoxic T cells (TC), and natural killer (NK) cells, as well as to facilitate the identification of new biomarkers and therapeutic targets of immunological and hematological diseases.

Introduction

Lymphocytes express on their surface a distinct constellation of molecules, many of which are used to characterize the several stages of their lineage-specific differentiation or their different states of activation or inactivation.

As surface antigens, CD molecules function in a number of different ways, often acting as receptors or ligands important to cell-cell communication. These receptors process and convey external signals into the cell by activating complex signaling cascades which ultimately alter the behavior of the cell (e.g. promote growth, differentiation, and isotype switching or cytokine production). Some CD antigens do not play a role in cell signaling, but have other functions, including adaptive immunity, cell adhesion, and platelet adhesion and platelet aggregation.

Over the past decades, CD antigens have become attractive and reliable markers for the identification and characterization of lymphocyte populations and sub-populations, a technique referred to as immunophenotyping. This method utilizes fluorochrome-labeled monoclonal antibodies (mAbs) reactive against CD markers, and together with the advances in multicolor flow cytometry, have been paramount in determining their expression and function. However, because the type of CD antigen and the degree to which it is expressed varies drastically amongst lymphocyte populations, using a combination of CD antibodies can maximize the resolution of a particular maker lineage or sub-lineage population (See table 1).

In biomedical research, characterizing the dynamic expression of CD antigens in correlation to different pathophysiological conditions has become instrumental in the diagnosis and treatment of leukemia's, lymphomas and immune system disorders such as autoimmune disease.

For example, the therapeutic mAb orthoclone OKT3 (also known as Muromonab-CD3) is an immunosuppressive agent used to reduce acute rejection in patients receiving organ transplants such as liver or kidney.

T Lymphocytes and Their Subsets

T lymphocytes, which originate in the bone marrow and then migrate to the thymus for maturation, are key players of the adaptive immune system. Together with B lymphocytes, these cells orchestrate a variety of immune-related functions (e.g. eliminating infected host cells, activating additional immune cells, producing cytokines, secreting antibodies, etc.) that ultimately shape the landscape of the immune response.

Differentiating T lymphocyte populations and subsets is rather straightforward since many key surface markers have already been conveniently identified and a broad selection of CD antibodies validated for their detection are commercially available.

Identifying Cytotoxic and Helper T Cell Populations

CD3, which is T cell co-receptor involved in the activation of two major T cell populations, cytotoxic T cells and helper T cells, is universally expressed on all mature T lymphocytes (CD3+). The specificity of CD3 for T lymphocytes and its appearance throughout T cell maturation makes it a defining feature and identifying marker of the T cell lineage.

In tandem with the T cell receptor (TCR), CD3 will recognize and bind to either a peptide-MHC class I or class II complex on antigen presenting cells (APCs). Along with the TCR/CD3 complex, each of the two major T cell populations expresses co-receptors which bind to the peptide-MHC complex and enhance T-cell responses.

CD4 co-receptors, which are expressed on the surface of helper T cells and facilitates their activation, binds to peptide-MHC class II complexes, whereas CD8 co-receptors, which are expressed on the surface of cytotoxic T cells, binds to peptide-MHC class I complexes.

Through immunophenotyping, fluorescently-labeled CD antibody conjugates can be used to identify the expression of these co-receptors and distinguish between these two

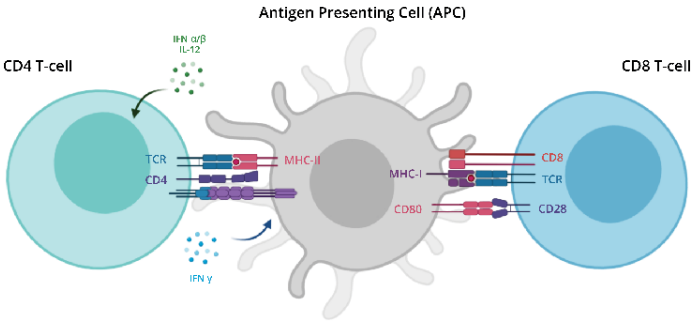


Figure 1. Illustration of CD4 and CD8 T cell activation (figure created in BioRender).

Table 1. Common CD markers used for the differentiation of leukocytes by flow cytometry.

Cell Type	Common Human CD Markers	Common Mouse CD Markers
B Cell	CD19, CD20	CD45R/B220, CD19, CD 22 (B cell activation marker)
Dendritic Cell	CD11c, CD123	CD11c, CD123
Endothelial Cell	CD146	CD146 MECA-32, CD106, CD31, CD62E (activated endothelial cells)
Epithelial Cell	CD326	CD326 (EPCAM1)
Erythrocyte	CD235a	CD235a, Ter-119
Granulocyte	CD66b	CD66b, Gr-1/Ly6G, Ly6C
Monocyte/Macrophage	CD14, CD33	CD11b/Mac-1, Ly-71 (F4/80)
Natural Killer (NK) Cell	CD56	CD335 (NKp46)
Platelet	CD41, CD61, CD62	CD41, CD61 (Integrin B3), CD9, CD62P (activated platelets)
Stem Cell/Precursor	CD34 (hematopoietic stem cell only)	CD34 (hematopoietic stem cell only)
T Cell	CD3, CD4, CD8	CD3, CD4, CD8

Table 2. Overview of monoclonal anti-human CD3, CD4 and CD8 antibodies.

Antibody	Immunogen	Class	Species Reactivity	Host, Isotype	Clone
Anti-human CD3	Recognizes CD3?-chain	Monoclonal	Human	Mouse, IgG1	HIT3b
Anti-human CD3	Recognizes CD3?-chain	Monoclonal	Human	Mouse, IgG1, kappa	SK7
Anti-human CD3	Recognizes CD3?-chain	Monoclonal	Human	Mouse, IgG1, kappa	UCHT1
Anti-human CD3	Recognizes CD3?-chain	Monoclonal	Human	Mouse, IgG2a, kappa	HIT3a
Anti-human CD3	Recognizes CD3?-chain	Monoclonal	Human	Mouse, IgG2a, kappa	OKT-3
Anti-human CD4	Recognizes CD4	Monoclonal	Human	Mouse, IgG1, kappa	RPA-T4
Anti-human CD4	Recognizes CD4	Monoclonal	Human	Mouse, IgG1, kappa	SK3
Anti-human CD4	Recognizes CD4	Monoclonal	Human	Mouse, IgG2b, kappa	OKT-4
Anti-human CD4	Recognizes CD4	Monoclonal	Human	Mouse, IgG2b, kappa	HIT4a
Anti-human CD8	Recognizes CD8?	Monoclonal	Human	Mouse, IgG1, kappa	HIT8a
Anti-human CD8	Recognizes CD8?	Monoclonal	Human	Mouse, IgG1, kappa	SK1
Anti-human CD8	Recognizes CD8?	Monoclonal	Human	Mouse, IgG2a	OKT-8

major T cell populations. CD3+CD4+CD8- counts are used to characterize helper T cells, and CD3+CD4-CD8+ counts are used to characterize cytotoxic T cells.

Designing a Multicolor Flow Cytometry Panel for T Lymphocyte Immunophenotyping

Lymphocytes express on their surface a distinct constellation of molecules, many of which are used to characterize the several stages of their lineage-specific differentiation or their different states of activation or inactivation.

As surface antigens, CD molecules function in a number of different ways, often acting as receptors or ligands important to cell-cell communication. These receptors process and convey external signals into the cell by activating complex signaling cascades which ultimately alter the behavior of the cell (e.g. promote growth, differentiation, and isotype switching or cytokine production). Some CD antigens do not play a role in cell signaling, but have other functions, including adaptive immunity, cell adhesion, and platelet adhesion and platelet aggregation.

Flow Cytometer Configuration and Fluorophore Selection

When designing a multicolor panel for flow cytometry it is important to check your instrument configuration as it can affect what fluorophores are available to you and how much compensation may be required before analyzing data. In multicolor flow cytometry, compensation is often necessary to account for the spectral overlap between fluorophores and correct for any spillover (i.e. when a signal from one fluorophore spills into the detectors of other fluorophores). While modern technology has made the compensation process much easier, choosing the right fluorophore can also minimize the need to perform compensation to begin with. To help determine fluorophore compatibility use a fluorescence spectra viewer to compare your instrument's configuration against the excitation and emission parameters of possible fluorophores.

The aforementioned 3-color panel was designed for an ACEA NovoCyte flow cytometer (ACEA Biosciences) equipped with a 488 nm laser and the appropriate filter sets (e.g. 530/30 nm, 585/40 nm and 670/40 nm filter sets). Using AAT Bioquest's interactive spectrum viewer, fluorophores iFluor™ 488, PE, and PerCP were analyzed for spectral compatibility (Figure 1).

Based on this configuration, all three fluorophores can be well-excited by the 488 nm laser, however, compensation will be required to account for iFluor™ 488 spillover into the PE channel and PE spillover into the APC channel (Table 3).

Balance CD Marker Expression & Fluorophore Brightness

Another important parameter to consider when performing multicolor flow cytometry is to match the expression levels of

the CD markers you wish to detect with an appropriately bright fluorophore. Primary CD markers are well-characterized and often densely expressed. These types of markers pair well with dim to moderately bright fluorophores. Tertiary markers, however, are poorly characterized and expressed at low levels, and pair well with moderate to bright fluorophores. In the case of CD3, CD4 and CD8 markers, which are all well-characterized, T lymphocytes have highly heterogenous CD3 expression compared to CD4 and CD8. Thus antibodies conjugated to PerCP, a relatively dim fluorophore, can be used to detect CD3.

For CD4 and CD8 detection, iFluor™ 488 and PE conjugated CD antibodies can be used, respectively. iFluor™ 488 is a moderately bright fluorophore and PE is a bright fluorophore.

For more information regarding fluorophore brightness, refer to our application note: Relative Brightness of Fluorescent Dyes.

Materials and Methods

PBMC Cell Preparation

Cryopreserved human PBMCs (10 million cells/vial, iXCells Biotechnologies Cat No. 10HU-003) were thawed and cultured immediately in order to retain the highest cell viability.

To thaw cells, place vial in a 37 °C water bath with gentle agitation for ~1 minute.

Pipette cells into a 15 mL conical tube with 10 mL fresh Blood Cell Culture Medium containing 10% Fetal Bovine Serum (i.e. Total Volume = 10.1 mL (100 µL PBMCs + 9 mL fresh Blood Cell Culture Medium + 1 mL Fetal Bovine Serum)). Then centrifuge at 1,000 rpm (~220g) for 5 minutes at room temperature and remove supernatant.

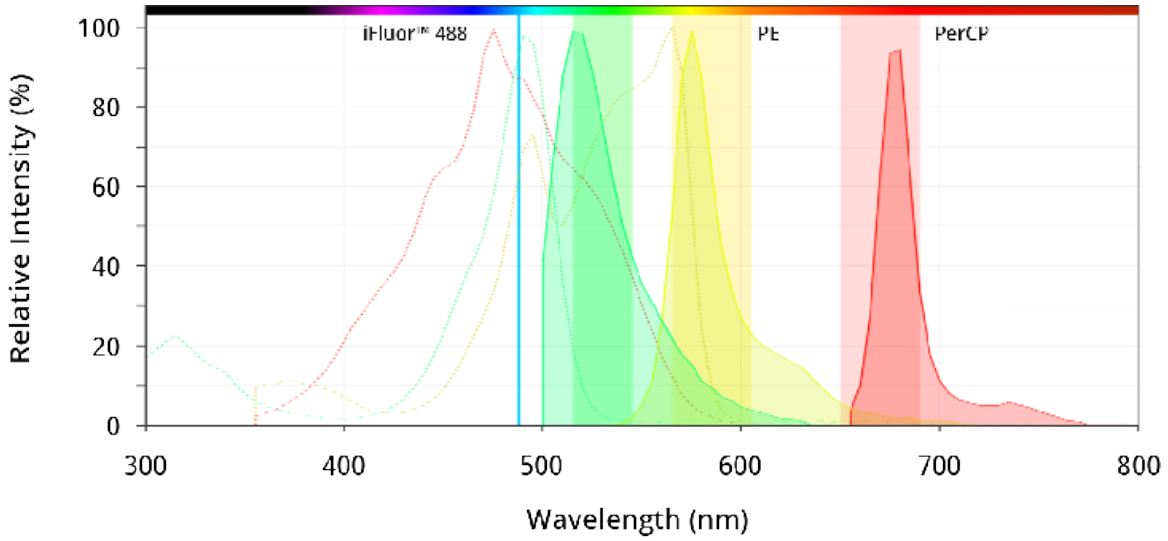


Figure 2. Representation of excitation and the overlapping emission spectra of iFluor™ 488 (Green, Cat No. 1023) PE (Yellow, Cat No. 2556) and PerCP (Cat No. 2559). Multicolor analysis may require compensation prior to data analysis. Compensation accounts for this overlap by removing signals that "spillover" into the main signal of interest.

Table 3. 3-Color multicolor flow cytometry panel.

Fluorophore	Ex (nm)	Em (nm)	Laser	Peak Intensity	Filters	Spillover (530/30 filter)	Spillover (585/40 filter)	Spillover (670/40 filter)
iFluor™ 488	491 nm	516 nm	Blue laser	96%	530/30	63%	16%	0%
PE	566 nm	574 nm	Blue laser	65%	585/40	0%	79%	5%
PerCP	477 nm	678 nm	Blue laser	87%	670/40	0%	0%	80%

PBMC Cell Staining

Prior to immunolabeling, determine PBMC health using a viability assay such as the Trypsan Blue Dye Exclustion Test (AAT Bioquest, Cat No. 2452). For each staining condition we recommend using 0.5-1x106 cells/sample. Wash PBMCs with HHBS buffer (AAT Bioquest, Cat No. 20011) 2 times by centrifuge at 1000 RPM for 5 minutes each wash.

Block non-specific Fc-mediated interactions by re-suspending PBMCs in Assay Buffer (HHBS buffer containing 1% BSA solution) with Human TruStain FcX™ (BioLegend, Cat No. 422302) 5 µL/100 µL of Assay Buffer and incubate at room temperature for 10 minutes. Incubate PBMCs with CD3/CD4/CD8 conjugate for 20 minutes in the dark, on ice. Wash PBMCs with Assay Buffer twice and then resuspend PBMCs in Assay buffer.

Sample is now ready to be analyzed on a flow cytometer equipped with a 488 nm laser.

Product	Unit Size	Cat No.
PerCP Anti-human CD3 Antibody	25 Tests	100301T0
PerCP Anti-human CD3 Antibody	100 Tests	100301T1
PerCP Anti-human CD3 Antibody	500 Tests	100301T2
PerCP Anti-human CD3 Antibody	25 Tests	100311T0
PerCP Anti-human CD3 Antibody	100 Tests	100311T1
PerCP Anti-human CD3 Antibody	500 Tests	100311T2
PerCP Anti-human CD3 Antibody	25 Tests	100321T0
PerCP Anti-human CD3 Antibody	100 Tests	100321T1
PerCP Anti-human CD3 Antibody	500 Tests	100321T2
PerCP Anti-human CD3 Antibody	25 Tests	100331T0
PerCP Anti-human CD3 Antibody	100 Tests	100331T1
PerCP Anti-human CD3 Antibody	500 Tests	100331T2
PerCP Anti-human CD3 Antibody	25 Tests	100341T0
PerCP Anti-human CD3 Antibody	100 Tests	100341T1
PerCP Anti-human CD3 Antibody	500 Tests	100341T2
iFluor™ 488 Anti-human CD4 Antibody	100 Tests	10040050
iFluor™ 488 Anti-human CD4 Antibody	500 Tests	10040051
iFluor™ 488 Anti-human CD4 Antibody	100 Tests	10041050
iFluor™ 488 Anti-human CD4 Antibody	500 Tests	10041051
iFluor™ 488 Anti-human CD4 Antibody	100 Tests	10042050
iFluor™ 488 Anti-human CD4 Antibody	500 Tests	10042051
iFluor™ 488 Anti-human CD4 Antibody	100 Tests	10043050
iFluor™ 488 Anti-human CD4 Antibody	500 Tests	10043051

A Practical Guide for

Labeling Antibodies

Introduction

Labeling antibodies with a specific, detectable tag is an essential technique for many biological research fields. The labels used range from fluorescent dyes to hapten molecules like biotin, and many more, with enzyme tags gaining popularity in recent years for use in assays. The labels are generally composed of 2 moieties: the functional group for binding, and the tag (such as a fluorophore) for detection. Labeled antibodies are used to visualize protein-protein interactions, immune activity, and many other cellular behaviors. The choice of label typically depends on the application. Enzymatic tags are typically used for enzyme-linked immunosorbent assays (ELISAs) and immunohistochemistry (IHC), while fluorescent labels are used for flow cytometry, immunofluorescence (IF), and general cellular imaging. Hapten tags can be used in protein isolation and purification, among other applications. Regardless of which downstream application the labeled antibodies will be used for, precise preparation and material selection are the keys to an efficient, reproducible workflow.

Antibody Structure

Antibodies are large Y-shaped proteins, with Immunoglobin G being the most common type. An antibody is made up of 4 peptide chains: 2 identical light, or L, chains and 2 identical heavy, or H, chains. The top of the Y is the variable region, and the tips of each Y are the binding sites. The upper segment of the Y is the fragment antigen binding region (Fab), and the bottom of Y is the constant part, specifically termed the fragment crystallizable region (Fc). The steady-state Fc region is what interacts with the surface of the cell during immune system activation. The very center is called the hinge region, and simply refers to the disulfide bonds that hold the antibody together.

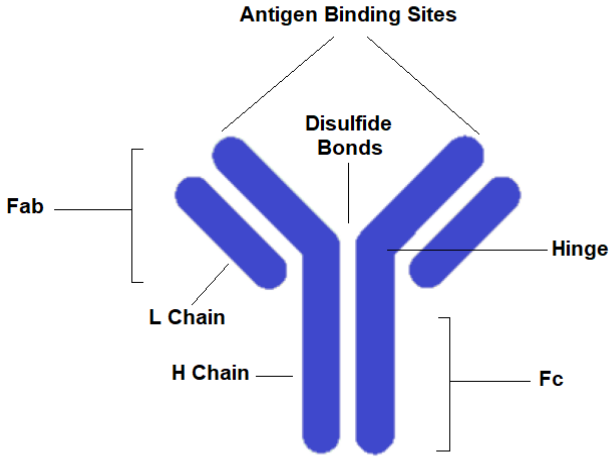


Figure 1. Comparison of DNA detection in 1% agarose gel in TBE buffer using Gelite™ Safe, EtBr, and SYBR® Safe. Two-fold serial dilutions of 1 kb DNA ladder were loaded in amounts of 100 ng, 50 ng, and 25 ng from left to right. Gels were stained for 60 minutes with Gelite™ Safe, EtBr, and SYBR® Safe according to the manufacturer's recommended concentrations and imaged using the ChemiDoc™ Imaging System (Bio-Rad®). Gels were illuminated using a 300 nm transilluminator fitted with GelGreen and GelRed filters.

Types of Antibody Labeling and Their Uses

The 2 primary types of antibody labeling are amine-reactive and thiol-reactive labels, named for which area of the antibody is to be modified.

Amine-Reactive Dyes

Amine-reactive labels target primary amines (also called lysine groups) that are interspersed throughout the peptide chains of the antibody. This method is the most popular because of the prevalence of these (-NH2) groups in most antibodies, making it an effective default choice. The reaction is stable above a pH level of 7.5, the default concentration is a simple 1mg/mL, and the equipment requirements are minimal, making it an excellent choice for most laboratories.

Reflective of this popularity, there are many amine-reactive fluorescent labels available commercially, with the most popular choice being succinimidyl esters (SE). Well-known examples include Thermo Fisher's Alexa Fluor® and AAT Bioquest's iFluor™ SE dye series.

This type of labeling is used for many biomolecules aside from antibodies, including peptides and nucleic acids, for applications ranging from fluorescence in situ hybridization (FISH) to immunohistochemistry (IHC). A potential drawback of this labeling method is a tendency towards poor shelf life, necessitating immediate use of the resulting conjugate.

This method can be challenging to optimize, since under- or over-labeling the antibody will affect the performance of the resulting conjugate.

Thiol-Reactive Dyes

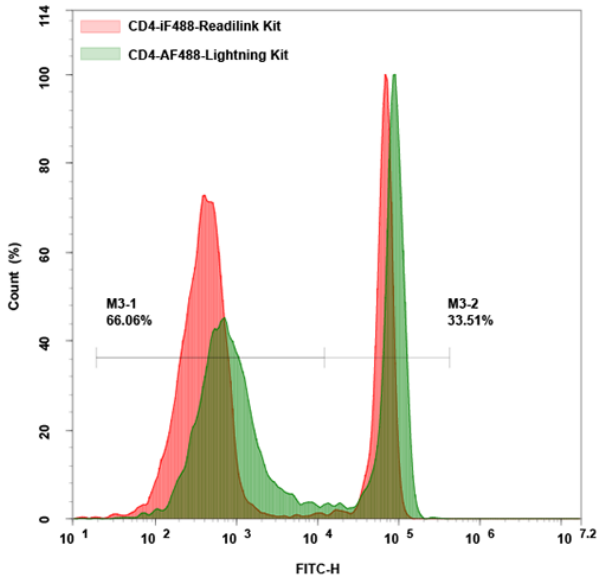
Thiol-reactive labeling targets the sulfhydryl bonds (-SH) on the outer portions of antibodies. This method is popular when conjugating larger molecules like phycoerythrin (PE), alkaline phosphatase (AP), or allophycocyanin (APC). AAT Bioquest has a guide available specifically for PE and APC conjugate applications, which have specific challenges.

The appeal of this method for larger conjugates is that the antibody binding sites at the upper tips of each Y are left open. By not interfering with these binding sites, researchers can minimize the effect of labeling on the activity and viability of the antibody.

Additionally, since these bonds only occur in specific locations, thiol-reactive labeling is an ideal choice if site-specific, controlled conjugation is required. There are multiple thiol-reactive labels available, with a popular variety being maleimides, used after the application of a reducing agent such as tris(2-carboxyethyl)phosphine (TCEP) or dithiothreitol (DTT). Specifically for large enzyme conjugation, techniques such as SMCC crosslinking are typically required.

These compounds have a maleimide group on one end and an amine-reactive group such as an NHS ester on the other. Although effective, this method requires extensive biochemical background to optimize, and can have very low yields. Modern kits, such as the Buccutite™ series, bypass this requirement as well as attendant purification steps, allowing 100% recovery of the resulting conjugates.

For a detailed example protocol for maleimide labeling, consult the 'Labeling Proteins with iFluor™ Dye Maleimides' application note.



Labeling Kit	CD4(SK3)- Conjugate	Stain Index
ReadiLink™ Kit	CD4-iFluor™ 488 Conjugate	43
Lightning-Link® Kit	CD4-Alexa Fluor® 488 Conjugate	26

Figure 2. Flow cytometry analysis of human PBMC cells. Anti-human CD4 antibody prepared with ReadiLink™ Rapid iFluor488 Antibody Labeling Kit (Cat No. 1255) or Lightning-Link® Rapid Alexa Fluor ® 488. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the FITC channel.

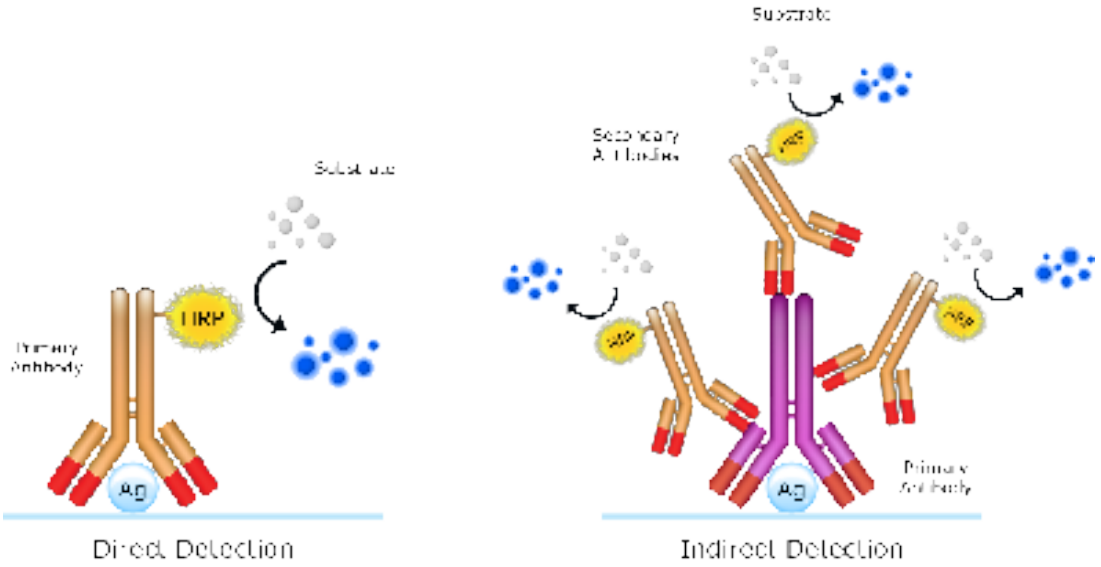


Figure 3. Simplified illustration of direct vs. indirect antibody detection mechanisms. The target antibodies are each shown attached to the experimental surface (shown as a blue line) such as in common microplate assays such as ELISA. HRP has been chosen in this example as a common label. On the left is the direct detection mechanism, with an HRP-conjugated primary antibody reacting with a colorimetric substrate. On the right is the indirect detection mechanism. The target antibody has been bound by multiple HRP-conjugated secondary antibodies, each of which reacts with the colorimetric substrate, giving a more intense signal.

Antibody Labeling Kits

For smaller labs, employing an antibody-labeling kit is usually preferred, for reasons of efficiency. It is often useful to compare the performance of 2 or more kits before the final selection, as results can vary between cell lines and antibody types. (See Figure 2 for an example of a comparison)

Detection of Labeled Antibodies

The 2 major categories of antibody detection are either direct (chemically binding a fluorophore or other traceable label to a target antibody) or indirect (conjugating a directly-labeled secondary antibody to the target antibody).

For highly-expressed targets, direct detection is usually employed, since it is simple and efficient. For rarer targets, indirect detection is preferred due to the option of amplifying the signal with multiple labels per target.

Tyramide signal amplification (TSA) is a popular option for improving low-abundance readings, recently superseded by Power Styramide™ signal amplification (PSA™). Indirect detection varies widely based on the downstream application, and developing optimized protocols, including all necessary positive and negative controls to verify specific binding, is a time-consuming process. Many researchers prefer to adapt known procedures, adjusting as necessary.

Not only does this method benefit from high sensitivity and specificity, but it also allows multiplexing qPCR using probes with different combinations of reporter dyes. This allows for an increase in throughput, meaning multiple samples can be assayed per plate, and consequently, there is a reduction in both sample and reagent usage.

Direct Antibody Detection

Many techniques, including immunofluorescence and immunohistochemistry, use direct detection, which gives quick, effective visualization of most targets. The conjugated antibody provides a signal in reaction to stimulus (in the form of an added substrate or wavelength of light) commensurate with the amount

of antibody present. Each antibody can only be linked with one reporter molecule, which makes quantification simple, but also means that the signal cannot be enhanced.

For rare targets, this can mean that the lower signal can be difficult to detect.

Indirect Antibody Detection

Indirect detection is an extension of direct detection, with secondary, tag-conjugated antibodies used in sequence with the primary target antibody.

The general procedure is largely the same, with the protocols being determined by which portion of the primary antibody will be targeted (amine, thiol, or other type), and then the selection of the secondary antibody that will provide the experimental signal.

Secondary antibody selection is a major component of successful protocols. Ideally, the secondary antibody will be highly specific to the target (minimizing general protein binding

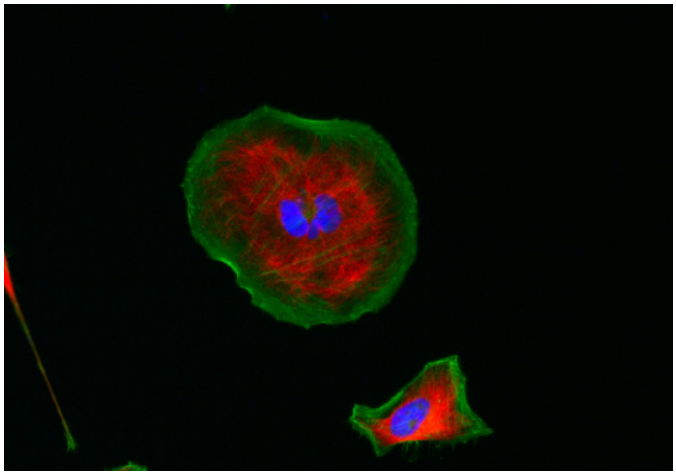


Figure 4. Example of fluorophore-labeled antibodies used to visualize cellular structure via direct detection. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 555 goat anti-mouse IgG (H+L) (red); actin filaments were stained with Phalloidin-iFluor™ 488 conjugate (green); and nuclei were stained with DAPI (blue).

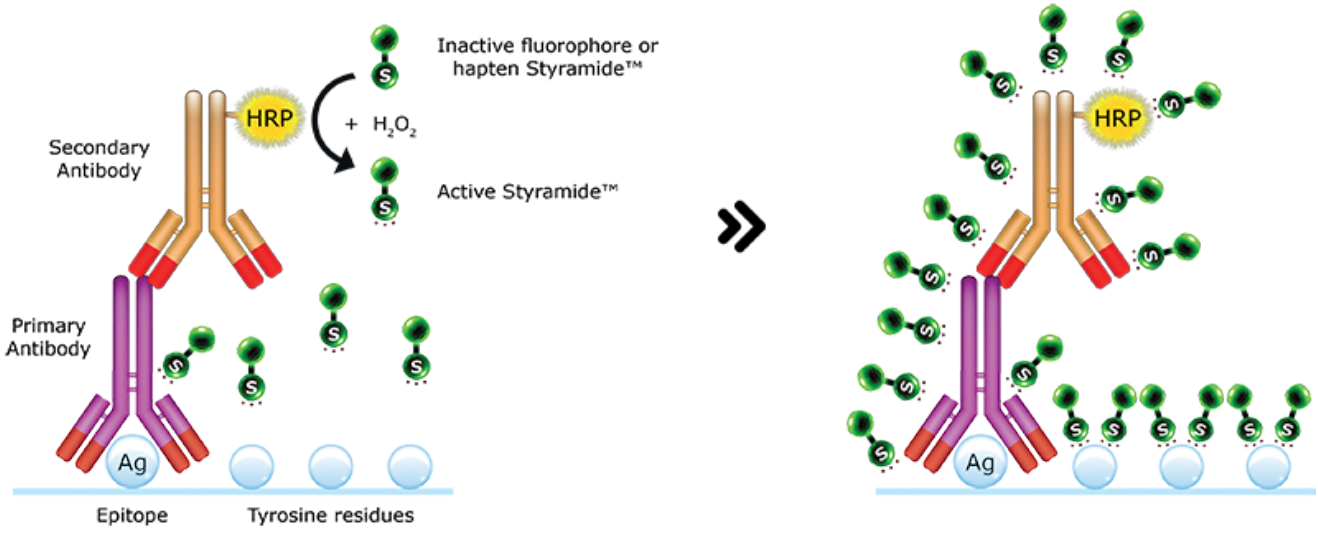


Figure 5. Simplified illustration of indirect detection and amplification, using PSA™ as an example. The primary antibody is shown at the bottom of each figure, colored in purple. The HRP-secondary conjugate (colored gold) is then bound to the primary target. HRP catalyzes the conversion of labeled inactive Styramide™ into highly-reactive Styramide™ radicals that covalently bind to tyrosine residues on and proximal to the enzyme site. The detectable signal is thus amplified exponentially.

and the resulting undesirable background noise) and be able to bind at multiple points on the target (to amplify the signal via multiplicative conjugation). This signal amplification is crucial for highly-sensitive assays and very low-abundance targets.

The secondary antibody chosen must also be conjugated to the correct reporter tag (enzyme, fluorophore, etc.) as needed by the experiment.

One major advantage of indirect detection is the ability to conjugate multiple reporters to a single primary target via multiple secondary antibodies conjugated to different tags. The downstream application (Flow cytometry, western blot, etc., will determine the optimal tags chosen.

Avoiding Common Difficulties and Challenges

Be mindful of downstream uses of the antibody. For amine-reactive direct labeling techniques, the TCEP reducing agent is incompatible with many assays, due to excessive background noise or other undesirable reactions. Use the ReadiUse™ TCEP removal solution or a similar alternative in order to eliminate residual TCEP in the reaction mixture.

Optimize antibody concentration. The antibody concentration recommended is at least 1mg/ml for most antibody labeling protocols, but can be modified as necessary if results are suboptimal. For experiments where the antibody availability is very low, using an antibody labeling kit that does not require a purification step (allowing 100% retrieval) is recommended. See this table for options.

Select buffers with care. Any buffer that contains amino acids, such as Tris buffer, will react with labels such as SE dyes. A very common mistake is not ensuring that buffers are free of BSA and gelatin. These proteins will also react with most labels, leading to significant loss of material. Consult provided protocols, but if no buffer is specified, the assumed default is typically a physiological buffer such as PBS or acetate. For buffer preparations and recipes, see 'Additional Resources' below or use the AAT Bioquest Buffer Preparations and Recipes resource.

When selecting a secondary antibody, match the host species, class, and subclass of the primary antibody. In order to successfully bind to the target antibody, the secondary antibody must be raised against both the host and target species. For example, if the primary monoclonal antibody was raised in goats, then the ideal secondary antibody would be raised in a species other than goat, such as a rabbit anti-goat IgG. Polyclonal IgG antibodies will be recognized by anti-IgG antibody H+L (heavy and light chains). Consult our Secondary Antibodies selection page for more in-depth information.

Include all necessary controls. These include both positive and negative controls, with others as needed. For antibody labeling, in order to verify accurate, specific binding, and to minimize detection errors, there are multiple varieties. These include isotype controls, (primary antibodies included to confirm that the binding of primary antibodies is specific to the target of interest) unstained controls, (experimental sample without labeling, to determine base autofluorescence from the cells themselves) and antibody-isolate controls (to determine what degree of antibody binding is occurring with other proteins present in the sample). Others are often necessary in order to establish confidence in experimental results.

Additional Resources

AAT Bioquest Degree of Labeling Calculator

This is an open-access online tool for determining the number of dye molecules conjugated to a particular protein. The DOL is an important quantity when trying to optimize an experiment. Typically, too many dyes attached to a protein will result in a self-quenching effect, reducing the net fluorescence of the conjugate. On the other hand, insufficient labeling with too few dyes will also result in suboptimal fluorescence intensity. The DOL Calculator can be used to assess the optimal conjugation ratio.

A practical guide for use of PE and APC in flow cytometry

This AssayWise article provides a general overview of what phycoerythrin (PE) and allophycocyanine (APC) can provide in flow cytometry and FACS applications, and how to optimize their uses.

AAT Bioquest Buffer Preparations and Recipes

This is an open-access interactive resource of common buffers, cell media, and other solutions typically used in laboratories. The recipes are sorted by application, and include full instructions suitable for novice researchers.

On-Demand Webinar: Modern Methods for Fluorescent Antibody Labeling

This webinar is intended for researchers in both small- and large-scale laboratories, and covers common mechanisms and methods of bioconjugation, focusing on antibody fluorescent labeling, with an emphasis on the selection of appropriate techniques and materials.

References

1. Alam, M. K., El-Sayed, A., Barreto, K., Bernhard, W., Fonge, H., & Geyer, C. R. (2019). Site-specific fluorescent labeling of antibodies and diabodies using SpyTag/SpyCatcher system for in vivo optical imaging. Molecular
2. Kennedy, D., Cronin, U. P., Piterina, A., & Wilkinson, M. G. (2019). Heat and chemical treatments affect the viability, morphology, and physiology of Staphylococcus aureus and its subsequent antibody labeling for flow cytometric analysis. Applied and environmental microbiology, 85(17), e01006-19. https://doi.org/10.1128/AEM.01006-19
3. Sun, W., Huo, Y., Mei, Y., Zhou, Q., Zhao, S., & Zhuang, M. (2020) Identification of a Small Probe That Can Be Conjugated to Proteins by Proximity Labeling. ACS Chemical Biology. 15 (1), 39-43 https://doi.org/10.1021/acschembio.9b00842
4. Tertel, T., Bremer, M., Maire, C., Lamszus, K., Peine, S., Jawad, R., & Görgens, A. (2020). High?Resolution Imaging Flow Cytometry Reveals Impact of Incubation Temperature on Labeling of Extracellular Vesicles with Antibodies. Cytometry Part A. https://doi.org/10.1002/cyto.a.24034
5. Yu S, He X, Drelich A, et al. (2019). A practical strategy for immunofluorescent detecting multiple targets in mouse tissues without restrictions on the host specious resources of the primary antibodies. Pathology, Research and Practice. 215(5):1049-1053. https://doi.org/10.1016/j.prp.2019.02.013

Product	Unit Size	Cat No.
Helixyte™ Green Nucleic Acid Gel Stain *10,000X DMSO Solution*	1 mL	17590
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Helixyte™ Gold Nucleic Acid Gel Stain *10,000X DMSO Solution*	1 mL	17595
Gelite™ Green Nucleic Acid Gel Staining Kit	1 Kit	17589
Gelite™ Orange Nucleic Acid Gel Staining Kit	1 Kit	17594
Gelite™ Safe DNA Gel Stain *10,000X Water Solution*	100 µL	17700
Gelite™ Safe DNA Gel Stain *10,000X Water Solution*	500 µL	17701
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Gelite™ Safe DNA Gel Stain *10,000X Water Solution*	10 mL	17703
Gelite™ Safe DNA Gel Stain *10,000X DMSO Solution*	100 µL	17704
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Gelite™ Safe DNA Gel Stain *10,000X DMSO Solution*	1 mL	17706
Gelite™ Safe DNA Gel Stain *10,000X DMSO Solution*	10 mL	17707

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Korea:
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Website: http://www.cmscorp.co.kr

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Website: http://www.biosite.se

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Website: http://www.itk.nl

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Website: http://www.biosite.se

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Website: http://www.biomol.de

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Website: http://www.axilscientific.com

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Email: info@stratech.co.uk
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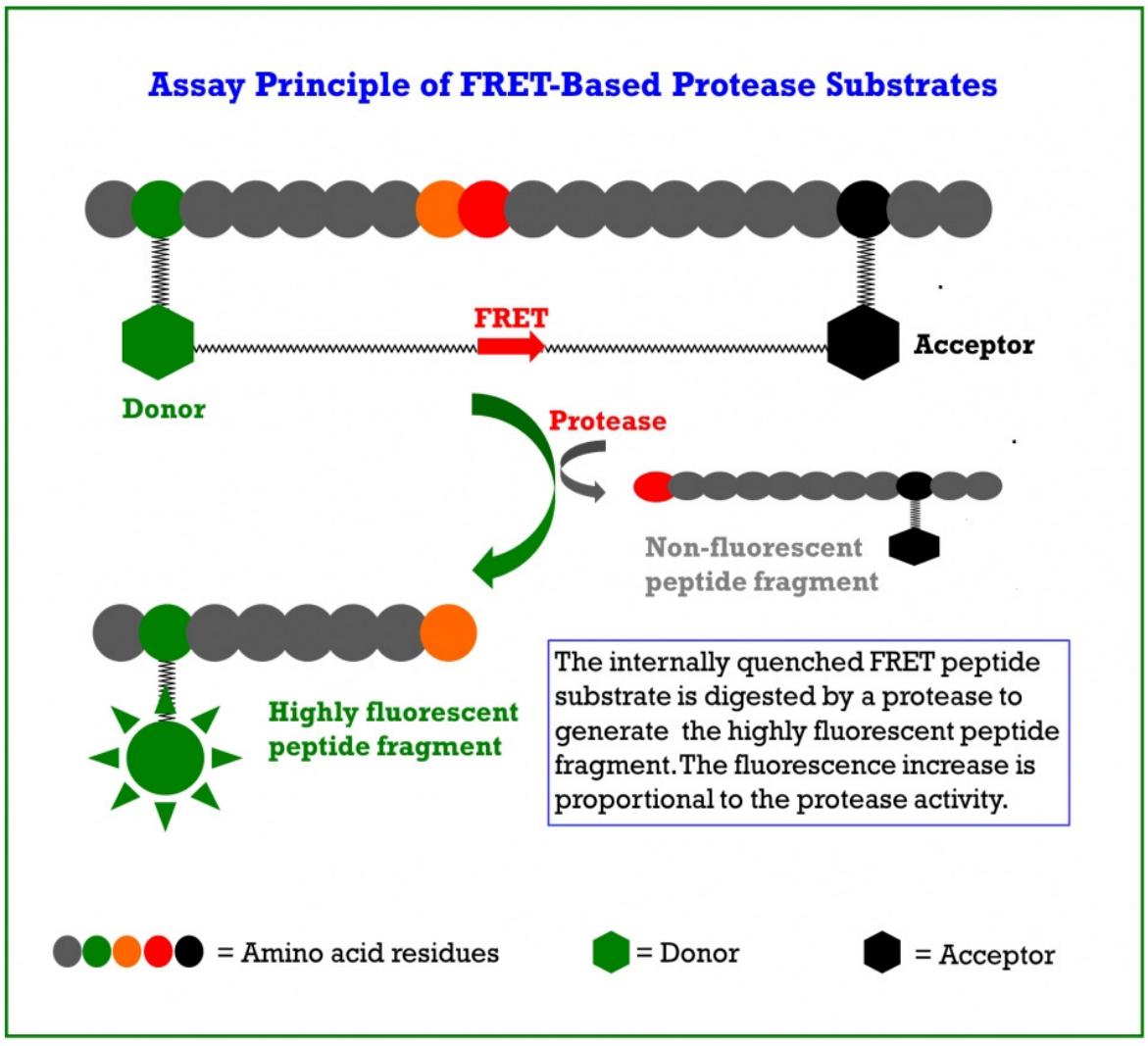
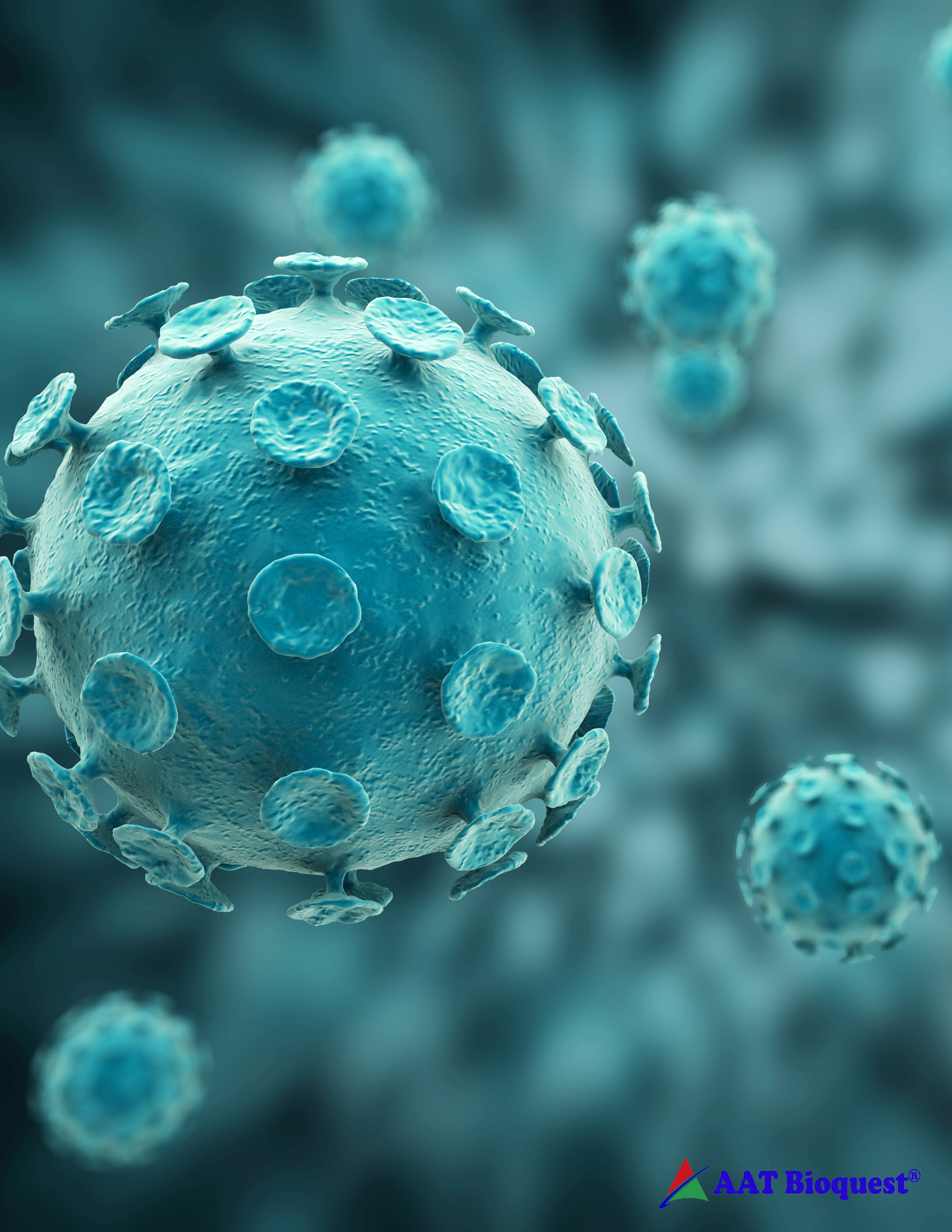
Principle of Covidyte & Covipyte Substrates

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