Streptavidin-Xtra™ IF488

Catalog number: 46000, 46001
Unit size: 100 ug, 1 mg

Component	Storage	Amount (Cat No. 46000)	Amount (Cat No. 46001)
Streptavidin-Xtra™ IF488	Freeze (< -15 °C), Minimize light exposure	1 vial (100 ug)	1 mg

OVERVIEW

Streptavidin conjugates are widely used in combing with biotin conjugates for detecting a variety of biological targets such as proteins, nucleic acids and other molecules. They are used as an ideal choice for many biological detections such as immunofluorescence microscopy, flow cytometry, western blot and other biological applications since streptavidin has a strong affinity binding biotin which is not affected over a broad range of pH and temperature. AAT Bioquest® offers a variety of streptavidin conjugates labeled with the classic fluorescent dyes (for example: FITC, TRITC, Texas Red®, Cy3®, Cy5® and Cy7®) and also our superior water soluble, photostable iFluor® and mFluor™ dyes. However, the conventional biotin-avidin detection systems are still limited by the limited signal intensity of the existing fluorescent conjugates. The Streptavidin Xtra™ iFluor conjugates are a new family of super bright streptavidin conjugates with nearly identical excitation and emission properties to Alexa Fluor fluorophores with 3~5 folds signal improvement. It is a set of powerful tools to detect low abundance targets in cell imaging or flow cytometry. iFluor® 488 is one of the most common green fluorescence colors for the FITC channel imaging.

KEY PARAMETERS

Flow cytometer

Emission	530/30 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC channel

Fluorescence microscope

Emission	FITC filter set
Excitation	FITC filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Streptavidin-Xtra™ IF488 stock solution (1 mg/mL)

1. Add 100 µL (For Cat# 46000) or 1 mL (For Cat# 46001) of ddH2O to the vial to make a 1 mg/mL stock solution.

   Note: This constituted stock solution will be in PBS with 0.2% BSA.

PREPARATION OF WORKING SOLUTION

Streptavidin-Xtra™ working solution

1. For IF, the suggested staining concentration is 1-5 ug/ml. For FACS, the suggested concentration is at 0.1-0.5 ug / 100 uL / million cells in the staining buffer.

   Note: PBS + 0.1% BSA can be used as a staining buffer.

   Note: For the best performance of each application, the optimal concentration of this reagent needs to be carefully determined.

   Note: The suggested working dilution is provided as a guide only. It is recommended that the users titrate the product in their tests using proper positive and negative controls.

SAMPLE EXPERIMENTAL PROTOCOL

1. Block and treat the samples with antibodies of interest as per the manufacturer's recommendations.
2. Add biotin-conjugated secondary antibody working solution in the samples at appropriate concentration and duration.

   Note: Please verify the compatibility and type of your biotin-conjugated antibody with the primary antibody used in the experiment. For example, If the primary antibody is a mouse antibody, then a goat anti-mouse antibody bound with biotin can be used for the assay.

3. Incubate the cells with Streptavidin-Xtra™ working solution at room temperature for 30 minutes to 1 hour. *Note:* Optimal time for incubation needs to be determined carefully.
4. Remove the working solution and resuspend the cells in your choice of buffer.
5. Take the image using the fluorescence microscope or record the intensity using flow cytometer.

EXAMPLE DATA ANALYSIS AND FIGURES

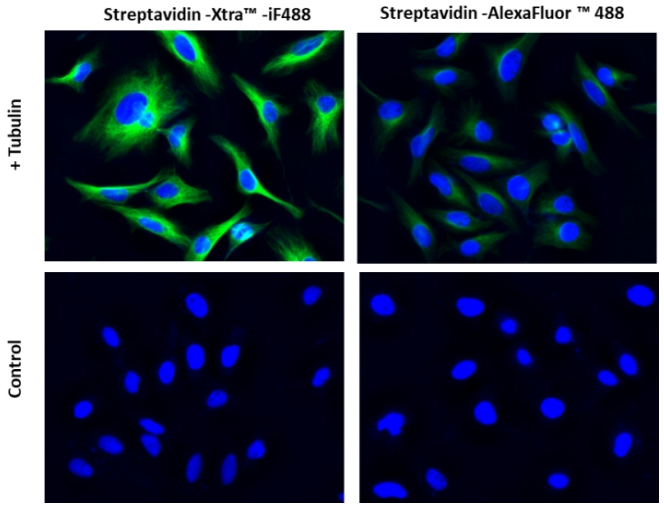

Figure 1. Images of Hela cells stained with Streptavidin-Xtra™ iFluor® conjugates and Streptavidin Alexa Fluor ™ conjugate. Hela cells were fixed with 4% paraformaldehyde for 30 minutes,

permeabilized with 0.02% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour. Fixed Hela cells were then stained with 1 µg/mL alpha Tubulin Mouse Monoclonal Antibody for 1 hour at room temperature, followed by GxM IgG-biotin (Cat# 16729) stain and then visualized with Streptavidin-Xtra™ iFluor 488 and Streptavidin-Alexa Fluor™ 488. Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17535).

DISCLAIMER

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