iFluor™ 647-streptavidin conjugate

Catalog number: 16966, 16996
Unit size: 200 ug, 1 mg

Component	Storage	Amount	
		Cat No. 16966	Cat No. 16996
iFluor™ 647-streptavidin conjugate	Freeze (<-15 °C), Minimize light exposure	200 ug	1 mg

OVERVIEW

AAT Bioquest's iFluor™ dyes are optimized for labeling proteins, in particular, antibodies. These dyes are bright, photostable and have minimal quenching on proteins. They can be well excited by the major laser lines of fluorescence instruments (e.g., 350, 405, 488, 555 and 633 nm). Streptavidin conjugates are widely used together with a conjugate of biotin for specific detection of a variety of proteins, protein motifs, nucleic acids and other molecules since streptavidin has a very high binding affinity for biotin. A variety of the complementary biotinylated reagents are available from numerous commercial vendors.This iFluor™ 647-streptavidin conjugate comprises streptavidin (as the biotin-binding protein) with iFluor™ 647 covalently attached (as the fluorescent label). It is commonly used as a second step reagent for indirect immunofluorescent staining, when used in conjunction with biotinylated primary antibodies. iFluor™ 647-streptavidin conjugates has fluorescence excitation and emission maxima of ~654 nm and ~674 nm respectively. These spectral characteristics make it an excellent alternative to Alexa Fluor® 647-streptavidin conjugate (Alexa Fluor® is the trademark of Invitrogen). It is a very valuable tool for biotin-streptavidin-based biological assays and tests.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	Cy5 filter set
Emission:	Cy5 filter set
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Cy5 filter set
Instrument:	Flow cytometer
Excitation:	640 nm laser
Emission:	660/20 nm filter
Instrument specification(s):	APC channel

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.

iFluor™ 647-streptavidin conjugate stock solution (1 mg/mL):
Add 200 µL (**Cat#16966**) and 1 mL (**Cat#16996**) ddH_2O to make stock solution.

Note Store at 2-6ºC and kept away from light. To extend the shelf-life of this product, add an equal volume of glycerol to make a final concentration of approximately 50% glycerol and store at -20ºC.

PREPARATION OF WORKING SOLUTION

iFluor™ 647-streptavidin conjugate working solution:
For IF, the suggested staining concentration is at 75-750 ng/mL. For FACS, the suggested concentration is at 100 ng-1 µg/million cells in 1 mL staining buffer.

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

Note The working solutions can be made in aqueous buffers.

SAMPLE EXPERIMENTAL PROTOCOL

1. Block and treat the samples with biotinylated antibodies of interest as per the manufacturer's recommendations.
2. Add iFluor™ 647-streptavidin conjugate working solution in the samples at appropriate concentration.
3. Incubate 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

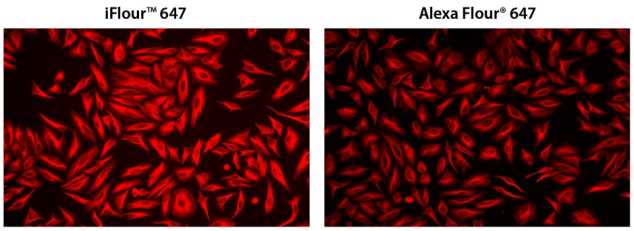

Figure 1. HeLa cells were incubated with mouse anti-tubulin and biotin goat anti-mouse IgG followed by AAT's iFluor™ 647-streptavidin conjugate (Red, Left) or streptavidin conjugated with Alexa Fluor® 647 (Red, Right), respectively.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

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