iFluor™ Ultra 647 succinimidyl ester

Catalog number: 71670, 71671, 71672
Unit size: 1 mg, 250 ug, 10 mg

Component	Storage	Amount (Cat No. 71670)	Amount (Cat No. 71671)	Amount (Cat No. 71672)
iFluor™ Ultra 647 succinimidyl ester	Freeze (< -15 °C), Minimize light exposure	1 mg	250 ug	10 mg

OVERVIEW

Fluorescent dye-conjugated antibodies provide a tool for identifying proteins in many applications including fluorescent cell imaging, flow cytometry, western blotting, immunohistochemistry and more. The advantages of using a fluorescently labeled antibody include higher sensitivity, multiplexing capabilities, and ease of use. iFluor™ Ultra family is a recent upgrade of our popular iFluor™ dyes and optimized for labeling antibodies used for fluorescence imaging and flow cytometry applications. Antibody conjugates prepared with iFluor™ Ultra 647 are far superior to the conjugates of other existing similar dyes such as Cy5, Dylight 650 and Alexa Fluor® 647. iFluor™ Ultra 647 conjugates are significantly brighter than the conjugates prepared with Cy5, Dylight 650 and Alexa Fluor® 647 under the same conditions. Additionally, the fluorescence of iFluor™ Ultra 647 is not affected by pH (4-10). iFluor™ Ultra 647 SE dye is reasonably stable and shows good reactivity and selectivity with protein amino groups. iFluor™ Ultra 647 has spectral properties and reactivity similar to Cy5, Dylight 650 and Alexa Fluor® 647 (Cy5® and Alexa Fluor® is the trademarks of GE Healthcare and ThermoFisher respectively).

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.

1. Protein stock solution (Solution A)
Mix 100 µL of a reaction buffer (e.g., 1 M sodium carbonate solution or 1 M phosphate buffer with pH ~8.5) with 900 µL of the target protein solution (e.g. antibody, protein concentration >2 mg/mL if possible) to give 1 mL protein labeling stock solution.

Note The pH of the protein solution (Solution A) should be 8.5 ± 0.5. If the pH of the protein solution is lower than 8.0, adjust the pH to the range of 8.0-9.0 using 1 M sodium bicarbonate solution or 1 M pH 9.0 phosphate buffer.

Note The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4. If the protein is dissolved in Tris or glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.

Note Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. The presence of sodium azide or thimerosal might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or spin column for optimal labeling results.

Note The conjugation efficiency is significantly reduced if the protein concentration is less than 2 mg/mL. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.

2. iFluor™ Ultra 647 SE stock solution (Solution B)
Add 100 µL high-quality, anhydrous dimethylsulfoxide (DMSO) or dimethyl-formamide (DMF) to 1 mg iFluor™ Ultra 647 SE to prepare 10 mg/mL stock solution. Mix well by pipetting or vortex.

Note Prepare the dye stock solution (Solution B) before starting the conjugation. Use promptly. Extended storage of the dye stock solution may reduce the dye activity. Solution B can be stored in freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

Note Once reconstituted, the NHS ester reactive dye solution is not very stable, especially if exposed to moisture. It could hydrolyze into the nonreactive free acid in aqueous solutions.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with iFluor™ Ultra 647 SE. You might need further optimization for your particular proteins.

Note Each protein requires distinct dye/protein ratio, which also depends on the properties of dyes. Over labeling of a protein could detrimentally affects its binding affinity while the protein conjugates of low dye/protein ratio gives reduced sensitivity.

Run conjugation reaction

1. Use 6-10 molar ratio of Solution B (dye)/Solution A (protein) as the starting point: Add 5 µL of the dye stock solution (Solution B, assuming the dye stock solution is 10 mM) into the vial of the protein solution (95 µL of Solution A) with effective shaking.

   Note We recommend to use 10:1 molar ratio of Solution B (dye)/Solution A (protein). If it is too less or too high, determine the optimal dye/protein ratio at 5:1, 15:1 and 20:1 respectively.

2. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

Purify the conjugation
The following protocol is an example of dye-protein conjugate purification with 15 mL MWCO=30K filter (https://www.emdmillipore.com/US/en/product/Amicon-Ultra-4-Centrifugal-Filter-Units,MM_NF-C7719)

1. Prepare column according to the manufacture instruction.
2. Load the reaction mixture (From "Run conjugation reaction") to the top of the column.
3. Add 4 mL of PBS (pH 7.2-7.4) as soon as the sample runs.
4. Centrifuge to concentrate to ~0.4 mL.
5. Add 4 mL PBS and then concentrate to ~0.4 mL.
6. Repeat ~3 times, until the elution absorbance at 650 nm < 0.1.
7. Collect the purified Antibody-iFluor™Ultra 647 conjugate solution.

EXAMPLE DATA ANALYSIS AND FIGURES

Characterize the Desired Dye-Protein Conjugate
The Degree of Substitution (DOS) is the most important factor for characterizing dye-labeled protein. Proteins of lower DOS usually have weaker fluorescence intensity, but proteins of higher DOS (e.g. DOS > 6) tend to have reduced fluorescence too. The optimal DOS for most antibodies is recommended between 2 and 10 depending on the properties of dye and protein. For effective labeling, the degree of substitution should be controlled to have 6-8 moles of iFluor™ Ultra

647 SE to one mole of antibody. The following steps are used to determine the DOS of iFluor™ Ultra 647 SE labeled proteins.

Measure absorption

To measure the absorption spectrum of a dye-protein conjugate, it is recommended to keep the sample concentration in the range of 1-10 μM depending on the extinction coefficient of the dye.

Read OD (absorbance) at 280 nm and dye maximum absorption (λmax = 656 nm for iFluor™ Ultra 647 dyes)

For most spectrophotometers, the sample (from the column fractions) need be diluted with de-ionized water so that the OD values are in the range of 0.1 to 0.9. The O.D. (absorbance) at 280 nm is the maximum absorption of protein while 656 nm is the maximum absorption of iFluor™ Ultra 647 SE. To obtain accurate DOS, make sure that the conjugate is free of the non-conjugated dye.

Calculate DOS

You can calculate DOS using our tool by following this link: https://www.aatbio.com/tools/degree-of-labeling-calculator

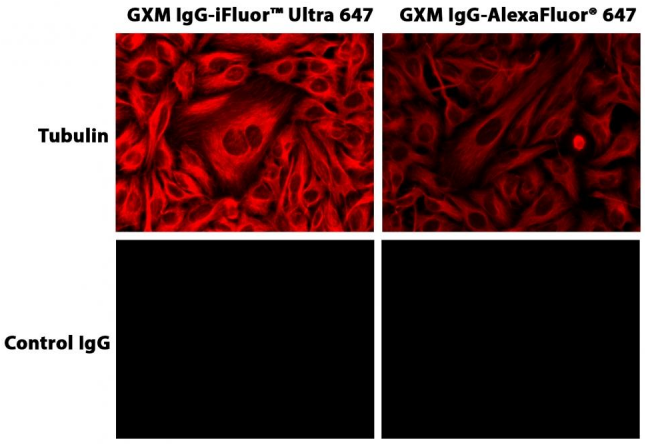

Figure 1. HeLa cells were incubated with mouse anti-tubulin followed by AAT's iFluor ™ Ultra 647 goat anti-mouse IgG conjugate or Alexa Fluor ® 647 goat anti-mouse IgG.

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.