iFluor® Ultra 488 succinimidyl ester

Catalog number: 71630
Unit size: 1 mg

Component	Storage	Amount (Cat No. 71630)
iFluor® Ultra 488 succinimidyl ester	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

iFluor® Ultra 488 succinimidyl ester is a green fluorescent dye designed for labeling primary amines ($R\text{-}NH_2$) on proteins, amine-modified oligonucleotides, and other amine-containing molecules. It is optimized for fluorescence microscopy and flow cytometry applications, providing high sensitivity and stability.

Fluorescent dye-conjugated antibodies are essential tools for protein detection in a wide range of applications, including fluorescence microscopy, flow cytometry, Western blotting, and immunohistochemistry. The use of fluorescently labeled antibodies offers several advantages, such as enhanced sensitivity, multiplexing capability, and streamlined workflows. The iFluor® Ultra series represents an advanced generation of iFluor® dyes, specifically engineered for antibody labeling in fluorescence imaging and flow cytometry.

iFluor® Ultra 488 exhibits superior photophysical properties compared to conventional green-fluorescent dyes, such as FITC, Oregon Green 488, and Alexa Fluor 488. Antibody conjugates prepared with iFluor® Ultra 488 display significantly greater fluorescence intensity under identical labeling conditions, potentially making it the brightest green-fluorescent dye for antibody conjugation. Additionally, its fluorescence remains stable across a broad pH range (4–10), ensuring consistent signal intensity in diverse experimental environments. The iFluor Ultra 488 SE variant demonstrates high stability, reactivity, and selectivity for protein amine groups, facilitating efficient conjugation. With spectral properties and reactivity comparable to FITC, Oregon Green 488, and Alexa Fluor 488, iFluor® Ultra 488 serves as a high-performance alternative for fluorescence-based assays requiring superior signal brightness and stability.

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

Protein stock solution (Solution A)

1. Mix 100 µL of a reaction buffer (e.g., 1 M sodium bicarbonate solution or 1 M phosphate buffer with pH ~8.5 to 9.0) 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. The final protein concentration range of 2-10 mg/mL is recommended for optimal labeling efficiency.

iFluor® Ultra 488 SE stock solution (Solution B)

1. Add anhydrous DMSO into the vial of iFluor® Ultra 488 SE to make a 10 mM 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 the freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

Add anhydrous DMSO into the vial of iFluor® Ultra 488 SE to make a 10 mM 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 the freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

Run conjugation reaction

1. Use a 10:1 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. The concentration of the protein is ~0.05 mM assuming the protein concentration is 10 mg/mL, and the molecular weight of the protein is ~200KD.

   Note: We recommend using a 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 by using a Sephadex G-25 column.

1. Prepare Sephadex G-25 column according to the manufacture instruction.

2. Load the reaction mixture (From "Run conjugation reaction") to the

top of the Sephadex G-25 column.

3. Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface.

4. Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired dye-protein conjugate.

   Note: For immediate use, the dye-protein conjugate must be diluted with staining buffer, and aliquoted for multiple uses.

   Note: For longer-term storage, the dye-protein conjugate solution needs to be concentrated or freeze-dried.

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® 488 SE to one mole of antibody. The following steps are used to determine the DOS of iFluor® Ultra 488 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 = 491 nm for iFluor® Ultra 488 dye)

For most spectrophotometers, the sample (from the column fractions) needs to be diluted with de-ionized water so that the O.D. 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 491 nm is the maximum absorption of iFluor® Ultra 488 SE. To obtain accurate DOS, ensure 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

DISCLAIMER