iFluor™ 790 succinimidyl ester

Catalog number: 1368, 71368, 71524, 71574
Unit size: 1 mg, 100 ug, 5 mg, 10 mg

Component	Storage	Amount (Cat No. 1368)	Amount (Cat No. 71368)	Amount (Cat No. 71524)	Amount (Cat No. 71574)
iFluor™ 790 succinimidyl ester	Freeze (< -15 °C), Minimize light exposure	1 mg	100 µg	5 mg	10 mg

OVERVIEW

In vivo fluorescence imaging uses a sensitive camera to detect fluorescence emission from fluorophores in whole-body living small animals. To overcome the photon attenuation in living tissue, fluorophores with long emission at the near-infrared (NIR) region are generally preferred, including widely used small indocarbocyanine dyes. Recent advances in imaging strategies and reporter techniques for in vivo fluorescence imaging include novel approaches to improve the specificity and affinity of the probes and to modulate and amplify the signal at target sites for enhanced sensitivity. Further emerging developments are aiming to achieve high-resolution, multimodality and lifetime-based in vivo fluorescence imaging. Our iFluor™ 790 is designed to label proteins and other biomolecules with near infrared fluorescence. Conjugates prepared with iFluor™ 790 have the excitation and emission spectra similar to that of indocyanine green (ICG) and the IRDye® 800 dye, with 783/814 nm excitation/emission maxima. iFluor™ 790 dye emission is well separated from commonly used far-red fluorophores such as Cy5, Cy7 or allophycocyanin (APC), facilitating multicolor analysis. This fluorophore is also useful for small animal in-vivo imaging applications or for other imaging applications that require NIR detections such as the two-color western applications with the LI-COR® Odyssey® infrared imaging system.

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 ~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. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.

2. iFluor™ 790 SE stock solution (Solution B)
Add anhydrous DMSO into the vial of iFluor™ 790 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 freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with iFluor™ 790 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 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 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 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 need be diluted with staining buffer, and aliquoted for multiple uses.

   Note For longer term storage, dye-protein conjugate solution need be concentrated or freeze dried.

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 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 2-3 moles of iFluor™ 790 SE to one mole of antibody. The following steps are used to determine the DOS of iFluor™ 790 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 = 803 nm for iFluor™ 790 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 803 nm is the maximum absorption of iFluor™ 790 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

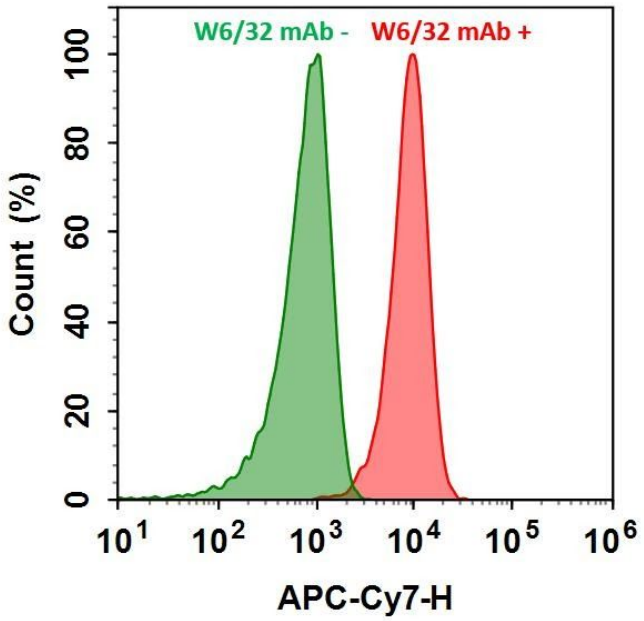

Figure 1.

HL-60 cells were incubated with (Red, +) or without (Green, -) Anti-human HLA-ABC (W6/32 mAb), followed by iFluor™ 790 goat anti-mouse IgG conjugate. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in APC-Cy7 channel.

DISCLAIMER

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