# trFluor™ Eu succinimidyl ester *europium complex*

Catalog number: 1433
Unit size: 1 mg

| Component | Storage | Amount (Cat No. 1433) |
|---|---|---|
| trFluor™ Eu succinimidyl ester | Freeze (< -15 °C), Minimize light exposure | 1 vial (1 mg) |

## OVERVIEW

Many biological compounds present in cells, serum or other biological fluids are naturally fluorescent, and thus the use of conventional, prompt fluorophores leads to serious limitations in assay sensitivity due to the high background caused by the autofluorescence of the biological molecules to be assayed. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes prompt fluorescence interferences. Our trFluor™ Eu probes enable time-resolved fluorometry (TRF) for the assays that require high sensitivity. These trFluor™ Eu probes have large Stokes shifts and extremely long emission half-lives when compared to more traditional fluorophores such as Alexa Fluor or cyanine dyes. Compared to the other TRF compounds, our trFluor™ Eu probes have relatively high stability, high emission yield and ability to be linked to biomolecules. Moreover, our trFluor™ Eu probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies.

## 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 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.

### trFluor™ Eu succinimidyl ester stock solution (Solution B)

1. Add anhydrous DMSO into the vial of trFluor™ Eu succinimidyl ester 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

This labeling protocol is specifically optimized for the conjugation of goat anti-mouse IgG with trFluor™ Eu succinimidyl ester. Further optimization maybe required for other proteins.

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

### Run conjugation reaction

1. Use a 10:1 molar ratio of Solution B (dye)/Solution A (protein) as a 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 low 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 needs to 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.

## 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 trFluor™ Eu succinimidyl ester to one mole of antibody. The following steps are used to determine the DOS of trFluor™ Eu succinimidyl ester 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 = 346 nm for trFluor™ Eu succinimidyl ester dyes)**

For most spectrophotometers, the sample (from the column fractions) needs to 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 346 nm is the maximum absorption of trFluor™ Eu succinimidyl ester. 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

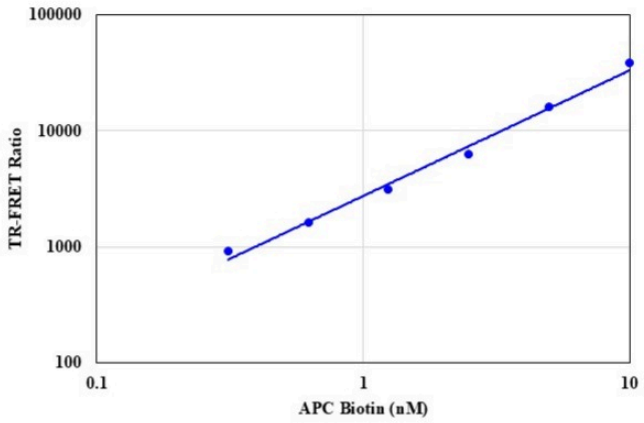


**Figure 1.** TR-FRET assay using Streptavidin-TrFluor™ Eu (#1433). The conjugate was prepared, and TR-FRET was detected in the presence of APC biotin (#3091).

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