trFluor™ Eu maleimide

Catalog number: 1434
Unit size: 100 ug

Component	Storage	Amount
trFluor™ Eu maleimide	Freeze (< -15 °C), Minimize light exposure	1 vial (100 ug)

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.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	346 nm
Emission	617 nm
Cutoff	370 nm
Recommended plate	Solid black

PREPARATION OF WORKING SOLUTION

Dye labelling solution (7mM)
Add 10 µL DMSO to the vial to make 7 mM dye labeling solution. **Note:** We recommend preparing fresh dye labelling solution.

SAMPLE EXPERIMENTAL PROTOCOL

Protocol for Labeling Proteins with trFluor™ Eu Maleimide:

1. Dissolve your thiol-containing protein at concentration 1-10 mg/mL (3-10 mg is the optimal labeling concentration) using PBS buffer (20 mM, pH 7.2).
2. Mix the trFluor™ Maleimide and protein solution at 20:1 molar ratio of dye/protein, and shake the reaction mixture at room temperature for 2-4 hours in the dark.
3. Filter the reaction mixture through a protein spin column for 100 µg to 1 mg protein labeling reaction; or purify the conjugate using gel filtration on a properly sized Sephadex G-25 column if the reaction scale is larger than 1 mg.
4. Collect the desired fractions for your immediate use or freeze dry them for your future use. **Note:** The trFluor™ conjugate need be used near neutral pH range (6.5 to 7.5). Either acidic or basic pH would reduce its fluorescence intensity.

Protocol for Labeling Small Molecules with trFluor™ Eu Maleimide:

1. Dissolve trFluor™ Maleimide (10 -15 mg/mL) and your thiol-contain molecule in DMSO at 1:1.2 molar ratio of dye/ thiol-contain molecule.
2. Stir the reaction mixture at room temperature for 2-4 hours in the dark.
3. Purify the conjugate using HPLC (ammonium acetate/water and acetonitrile, pH 7.0).
4. Collect and pool the desired fractions.
5. Combine and freeze-dry the pooled fractions. **Note:** The trFluor™ conjugate need be used near neutral pH range (6.5 to 7.5). Either acidic or basic pH would reduce its fluorescence intensity. **Note:** These protocols can be used as sample protovols. We recommend to modify as per needed.

EXAMPLE DATA ANALYSIS AND FIGURES

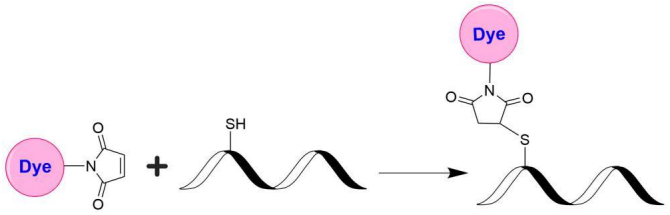

Figure 1.
Fluorescent dye maleimides are the most popular tool for conjugating dyes to a peptide, protein, antibody, thiol-modified oligonucleotide or nucleic acid through their SH group. Maleimides react readily with the thiol group of proteins, thiol-modified oligonucleotides, and other thiol-containing molecules under neutral conditions. The resulting dye conjugates are quite stable.

DISCLAIMER

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