trFluor™ HWS Eu maleimide *Europium complex*

Catalog number: 1448
Unit size: 100 ug

Component	Storage	Amount (Cat No. 1448)
trFluor™ HWS Eu maleimide *Europium complex*	Freeze (< -15 °C), Minimize light exposure	100 µg

OVERVIEW

trFluor™ HWS Eu maleimide is a europium-based fluorescent probe optimized for time-resolved fluorometry (TRF). Europium probes exhibit long emission lifetimes and large Stokes shifts, allowing delayed fluorescence detection that minimizes interference from short-lived autofluorescence commonly observed in biological samples. The HWS (Highly Water-Soluble) formulation provides significantly improved aqueous solubility compared to other similar commercially available europium dye complexes, supporting efficient conjugation and reduced aggregation during labeling reactions. The maleimide functional group reacts selectively with sulfhydryl groups on proteins, antibodies and other biomolecules to form stable covalent conjugates. These properties make trFluor™ HWS Eu maleimide suitable for sensitive immunoassays and other fluorescence-based detection systems that require low background and time-resolved measurement capability.

KEY PARAMETERS

Fluorescence microplate reader

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

PREPARATION OF WORKING SOLUTION

Dye labelling solution (7mM)

Add 10 µL DMSO to the vial to make ~5 mM dye labeling solution.

Note: We recommend preparing fresh dye labelling solution.

SAMPLE EXPERIMENTAL PROTOCOL

Protocol for Labeling Proteins with trFluor™ HWS 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™ HWS 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™ HWS 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™ HWS Eu Maleimide:

1. Dissolve trFluor™ HWS Eu 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™ HWS 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

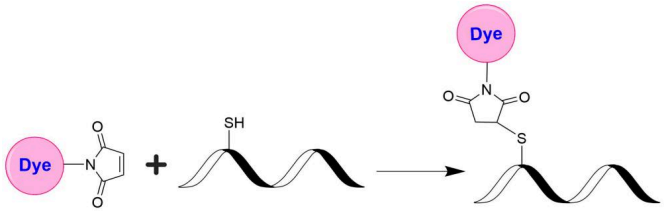

Figure 1. Fluorescent dye maleimides are the most popular tool for conjugating dyes to a peptide, protein, antibody, thiol-modified oligonucleotide or nucleic acid through their SH group. Maleimides react readily with the thiol group of proteins, thiol-modified oligonucleotides, and other thiol-containing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

DISCLAIMER

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