trFluor™ Eu Acceptor XL665

Catalog number: 1440
Unit size: 1 mg

Component	Storage	Amount (Cat No. 1440)
trFluor™ Eu Acceptor XL665	Minimize light exposure, Refrigerated (2-8 °C)	1 mg

OVERVIEW

trFluor™ Eu Acceptor XL665 is an excellent replacement for the commonly used D2 acceptor in labeling antibodies or antigens for bioconjugates paired with europium (Eu)-labeled probes in TR-FRET assays. Eu probes enable time-resolved fluorometry (TRF) in assays requiring high sensitivity. They exhibit large Stokes Shifts and exceptionally long emission half-lives compared to traditional fluorophores such as Alexa Fluor or cyanine dyes. In comparison to other TRF compounds, our TR Fluor™ Eu probes offer high stability, high emission yield, and the ability to be linked to various biomolecules. These probes serve as excellent donors for developing TR-FRET assays in conjunction with trFluor™ Eu Acceptor XL665. Since many biological compounds found in cells, serum, or other biological fluids are naturally fluorescent, using conventional, prompt fluorophores can significantly limit assay sensitivity due to the high background caused by the autofluorescence of the biological molecules to be assayed. Utilizing long-lived fluorophores combined with time-resolved detection, which introduces a delay between excitation and emission detection, minimizes prompt fluorescence interferences.

AT A GLANCE

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4. Purification (optional with this Buccutite method)

SAMPLE EXPERIMENTAL PROTOCOL

trFluor™ Eu Acceptor XL665 Conjugation Protocol with Antibody (SMCC Method)

Reduction of Antibody

1. Prepare a fresh 1.0 M DTT solution, by dissolving 15.4 mg of DTT in 100 µL of distilled water.

2. Prepare 0.7 mg of antibody, ensuring the concentration is at 1 mg/mL or higher for optimal results.

   Note: The reduction can be carried out in various buffers, such as MES, phosphate, or TRIS, with a pH range of 6 to 8.

3. Add 1.4 µL of 1.0 M DTT solution to 70 µL of the antibody solution and mix thoroughly. Allow the mixture to sit at room temperature for 30 minutes without further mixing to prevent cysteines from reoxidizing into cystines.

4. Purify the reduced antibody using a desalting column pre-equilibrated with 50 mM MES buffer (pH 6.0–6.5) supplemented with 2 mM EDTA.

5. Measure the antibody concentration using a Nanodrop spectrophotometer. Use the formula:

   Concentration (mg/ml) = A280nm / 1.4

   Note: Reduced antibodies are unstable. Perform the conjugation reaction immediately after purification to ensure optimal results.

Activate trFluor™ Eu Acceptor XL665:

1. Reconstitute trFluor™ Eu Acceptor XL665 in 100 µL ddH2O to make a 10 mg/mL.

   Note: The reconstituted trFluor™ Eu Acceptor XL665 can be stored at 4^{o}C for up to one month, protected from light.

2. Prepare a 10 mM stock solution of Sulfo-SMCC or SMCC in DMSO. Add 10–15 µL of the stock solution per 1 mg of trFluor™ Eu Acceptor XL665, mix thoroughly, and allow the reaction to proceed for 60 minutes.

3. Purify the activated trFluor™ Eu Acceptor XL665 using a desalting column that has been pre-equilibrated with 50 mM MES buffer (pH 6.0–6.5) containing 2 mM EDTA.

Reduced Antibody Conjugation with activated trFluor™ Eu Acceptor XL665:

1. Mix the reduced antibody directly with the pre-activated trFluor™ Eu Acceptor XL665 at the ratio of 1 mg trFluor™ Eu Acceptor XL665/0.7mg antibody.

   Note: If necessary, adjust the antibody amount proportionally to maintain this ratio.

2. Allow the reaction to proceed for 60–120 minutes at room temperature.

Purification

1. The antibody/trFluor™ Eu Acceptor XL665 conjugate can be further purified using size exclusion chromatography to achieve optimal performance.

   Note: Store the antibody/trFluor™ Eu Acceptor XL665 conjugate solution at 2–8°C, protected from light.

Reagents needed but not provided:

	Detailed Information	Recommended Ordering Information
DTT	DL-Dithiothreitol, MW=154.25 CAS# 3483-12-3	Sigma D0632
Sulfo-SMCC	Sulfo-SMCC [4-(N-Maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester, sodium salt] MW=436.37 CAS#: 92921-24-9	AAT Cat#4505 or equivalent
MES Buffer	50 mM MES Buffer (pH=6.0-6.5)+ 2 mM EDTA	
Desalting Column	ReadiUse™ Disposable PD-10 Desalting Column ReadiUse™ Bio-Gel P-6 Spin Column	AAT Cat#60504 AAT Cat#60500

trFluor™ Eu Acceptor XL665 Conjugation Protocol with Protein (Buccutite Method)

Note: This protocol uses an antibody as the protein of choice for the conjugation example. If you are conjugating trFluor™ Eu Acceptor XL665 with a different protein, adjust the protein quantity according to its molecular weight.

MTA Modification of Antibody:

1. Prepare a 10 mM stock solution of MTA, SE in DMSO.
2. Prepare 0.7 mg of antibody in PBS, ensuring the concentration is at 1 mg/mL or higher for optimal results.
3. Add 3.5 µL of 1.0 M $NaHCO_3$ buffer (pH 8.5) to 70 µL of the antibody solution and mix thoroughly. Then, add 4.5–7.5 µL of MTA, SE to the antibody solution, mix well, and incubate at room temperature for 60 minutes.
4. Purify the MTA-modified antibody using a desalting column that has been pre-equilibrated with 1X PBS.

FOL Modification of trFluor™ Eu Acceptor XL665:

1. Reconstitute trFluor™ Eu Acceptor XL665 in 100 µL ddH2O to make a 10 mg/mL.

   Note: The reconstituted trFluor™ Eu Acceptor XL665 can be stored at 4^{o}C for up to one month, protected from light.

2. Prepare a 10 mM FOL, SE stock solution in DMSO by adding 8–10 µL of a 10 mg/mL FOL, SE solution to 1 mg of trFluor™ Eu Acceptor XL665. Mix thoroughly and allow the reaction to proceed for 60 minutes.
3. Purify the FOL-activated trFluor™ Eu Acceptor XL665 using a desalting column pre-equilibrated with PBS buffer, such as the ReadiUse™ Bio-Gel P-6 spin column (Cat No. 60500).

MTA-Activated Antibody Conjugation with FOL-Activated trFluor™ Eu Acceptor XL665:

1. Mix the MTA-activated antibody directly with the pre-activated trFluor™ Eu Acceptor XL665 at the ratio of 1 mg trFluor™ Eu Acceptor XL665/0.7mg antibody.

   Note: If necessary, adjust the antibody amount proportionally to maintain this ratio.

2. Allow the reaction to proceed for 60–120 minutes at room temperature.

Purification (optional with this Buccutite method)

1. The antibody/trFluor™ Eu Acceptor XL665 conjugate can be further purified using size exclusion chromatography to achieve optimal performance.

   Note: Store the antibody/trFluor™ Eu Acceptor XL665 conjugate solution at 2–8°C, protected from light.

Reagents needed but not provided:

	Detailed Information	Recommended Ordering Information
MTA, SE	Buccutite™ MTA, NHS ester	AAT Cat#5355
FOL, SE	Buccutite™ FOL, NHS ester	AAT Cat#5350
NaHCO3 Buffer	1.0M NaHCO3 Buffer (pH=8.5)	
Desalting Column	ReadiUse™ Disposable PD-10 Desalting Column ReadiUse™ Bio-Gel P-6 Spin Column	AAT Cat#60504 AAT Cat#60500

EXAMPLE DATA ANALYSIS AND FIGURES

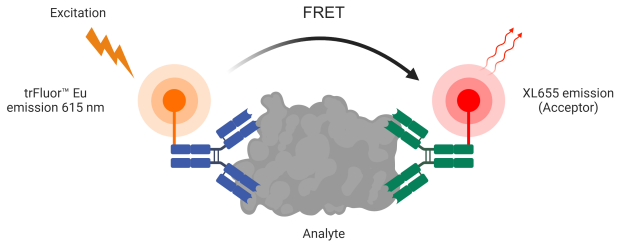

Figure 1. trFluor™ Eu Acceptor XL665 is an excellent replacement for the D2 acceptor that can be readily pair with Eu-labeled probes (such as TR Fluor™ Eu) for developing TR-FRET assays. TR-FRET assays are much more sensitive than the regular FRET assays that suffer serve interference caused by the naturally fluorescent compounds present in cells, serum or other biological fluids. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes prompt fluorescence interferences.