

GE Healthcare

Amersham Eu (TMT) Isothiocyanate

Product Booklet

Codes: PA99148 100 µg

PA99141 1 mg



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1. Legal

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TMT chelate is covered by U.S. Patent no. 5,367,080.

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2. Handling

2.1. Safety warnings and precautions

Warning: For research use only.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store lyophilized at 2–8°C. Following reconstitution the material may be stored at 2–8°C for up to 4 weeks, or at -15°C to -30°C in aliquots to avoid repeat freeze thaw cycles.

Each lyophilized component is stable for at least 12 weeks when stored under recommended conditions.

2.3. Expiry

The expiry date is stated on the pack label and will normally be at least 12 weeks from the date of despatch.

3. Component

Eu (TMT) Isothiocyanate, 1 mg or 100 µg.

4. Description

The products described are generic reagents that will allow the configuration of assays based on homogeneous time resolved fluorescence resonance energy transfer (TR-FRET).

Donor reagents have been labelled with a Terpyridine-bis (Methyl-enamine) Tetraacetic acid (TMT) chelate of europium (1).

CyTM5 has been used as the label for the acceptor reagents.

All reagents are available labelled with Eu (TMT) Isothiocyanate.

Evaluation of both donor and acceptor orientations will allow the optimization of assay configurations over a wide range of applications.

5. Protocol

5.1. Introduction

The need to screen large numbers of targets against extensive small molecule libraries has led to the demand for an increase in both the sensitivity and speed of assays. Increasingly assays based on fluorescence are becoming the method of choice.

Fluorescence resonance energy transfer (FRET) is the radiationless transfer of excitation energy from a donor to an acceptor molecule. This is a distance-dependent phenomenon occurring over 1–10 nm and as such is comparable with the dimensions of biological macromolecules. FRET is a valuable tool in studying proximity events in biological systems (2).

The majority of organic dyes, used for FRET, have a fluorescence lifetime of less than 10 ns.

Background fluorescence from biological samples, buffer components and plastic ware also have similar lifetimes. This can limit assay sensitivity (3). To overcome this, dyes with considerably longer lifetimes have been used. This technique, known as time resolved fluorescence (TRF) delays the measurement of assay signal until background fluorescence has dissipated. (Figure 1)

Time resolved fluorescence resonance energy transfer (TR-FRET) is a technique that combines the benefits of both FRET and TRF.

The fluorescence lifetime of chelates of rare earth elements is typically 100 μ s–2 ms, and offers advantages over conventional fluorophores as donors for TR-FRET. The use of Eu (TMT) as a donor and Cy5 as a spectrally matched acceptor will allow improvements in assay sensitivity, in particular the ratio of signal to background (4).

Principles of TRF (and TR-FRET)

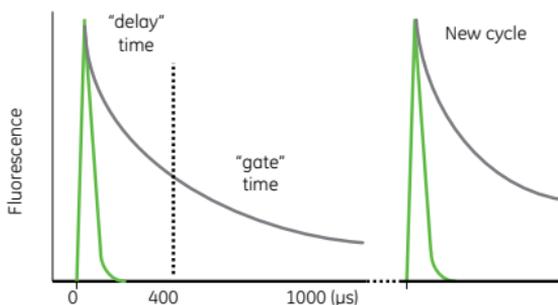


Figure 1. Principles of TRF and TR-FRET

GE Healthcare supply a range of donor and acceptor generic reagents that will allow researchers to perform sensitive, non-radiometric, screening assays using the TR-FRET technique.

5.2. Fluorescent labels

5.2.1. Europium (TMT) chelates

Lanthanide ions have a low extinction coefficient, and the presence of solvent, especially water, quenches their luminescence. Many organic ligands have been synthesized, which can 'chelate' a Lanthanide (III) ion, this diminishes the number of solvent molecules coordinated to the ion, allowing it to be sensitized to generate higher luminescence.

Effective ligands need to have a high coordination number, efficient light harvesting properties, long triplet excited state lifetime, large Stokes shift, low number of coordinated water molecules, solubility in aqueous media.

The aromatic terpyridyl structure (Figure 2), of Eu (TMT) provides the chelated Eu^{3+} ion with a relatively hydrophobic environment necessary for intense fluorescence output and long fluorescence

lifetime. Also the 3-amino group of the ligand is readily converted to the Isothiocyanate, this allows labelling of primary amine groups on proteins.

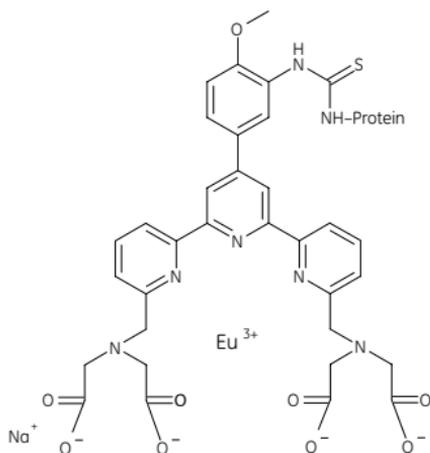


Figure 2. Eu (TMT) Isothiocyanate

The fluorescence lifetime of an aqueous solution of unconjugated Eu (TMT) Isothiocyanate was 1.4 ms.

The luminescence intensity of the Eu (TMT) was measured in either water or TRIS buffer in the presence of various additives. The addition of Ca^{2+} , Mg^{2+} and EGTA had no effect on intensity. However, when either Fe or Mn was added, the intensity dropped to zero almost immediately.

In buffers of varying pH little effect was seen on the emission spectra of Eu (TMT) ITC when excited at 340 nm (Figure 3). In addition, the presence of 10% DMSO had little or no effect on the emission spectrum or luminescence intensity observed.

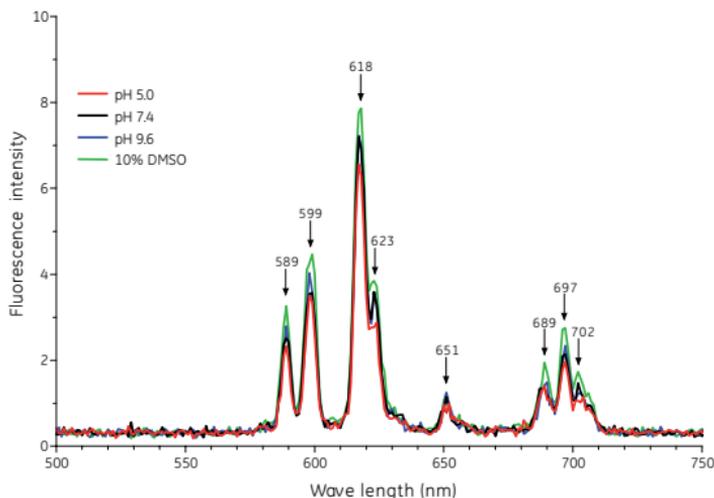


Figure 3. Grated emission spectra of Eu (TMT) ITC (unconjugated form)

5.3. Eu (TMT) Isothiocyanate

5.3.1. Reagent preparation

Lyophilized Eu (TMT) Isothiocyanate is available as either a 1 mg pack or a 100 µg pack.

Reconstitute to 10 mg/ml in DMSO/DMF [50:50] following reconstitution the material may be stored at 2–8°C for up to 4 weeks, or at -15°C to -30°C in aliquots to avoid repeat freeze thaw cycles.

5.3.2. Quality assurance

The labelled conjugates of Eu (TMT) have been tested in TR-FRET model assays, the resulting signal was measured using FARGe™ fluorescence plate reader with the following instrument settings:

Europium excitation	340 nm
Cy5 emission	670 nm
Delay (lag time)	50 µs
Signal integration	400 µs

Assay details and typical signal:background are shown below: This information should be used as a guide only and individual assay optimization is the responsibility of the end user.

5.4. Model assays

Example biological applications data has been generated using reagents from the product range.

This data should be used as reference information for customers wishing to establish their own applications. This information should be used as a guide only and individual assay optimization is the responsibility of the end user.

5.4.1. Antibody labelling using Eu (TMT) Isothiocyanate

Labelling of a Streptavidin molecule is carried out using 0.1 M Sodium Carbonate buffers at pH 9.0. The Streptavidin solution is transferred to the vial containing the weighed out Europium chelate labelling reagent, vortex mixed gently and placed on a roller mixer overnight at 2–8°C. Following incubation, the conjugate is transferred to fresh dialysis tubing (rinsed in the Super-Q water and the fresh PBS) and dialyzed in 10 L PBS for 1.5 hours at room temperature. This process is repeated once.

The conjugate is then transferred to PBS + 0.01% Sodium Azide and dialyzed for one hour at room temperature, transferred to fresh buffer and the dialysis repeated twice.

The dialyzed material is recovered and an accurate determination of the volume of material obtained. Material should be stored at 2–8°C in a sealed container and protected from light.

5.4.2. Oligonucleotide labelling using Eu (TMT) Isothiocyanate

Amine modified oligonucleotides should be freeze dried before labelling. Labelling is carried out in 0.1 M Sodium Carbonate buffer pH 9.0 at a concentration of approximately 0.5 μM . (Approximately 30–50 nmoles of oligo are labelled in a maximum volume of 200 μl of buffer.) Incubate the reaction mix overnight at 2–8°C with mixing. Purify the labelled oligonucleotide by PAGE.

6. Additional information

6.1. References

1. Toner, J. L. *et al.*, *J. Am. Chem. Soc.* **115**, 11032–11033 (1993).
2. Van Der Meer, B. W. *et al.*, *Resonance Energy Transfer, Theory and Data*, VCH Publishers, New York, ISBN 1-56081-643-0, (1994).
3. Sammes, P.G., *Nat. Prod. Rep.* **13**, 1–28. Review. (1996).
4. Jones, S. *et al.*, *Journal of Fluorescence* **11** (1), 13–21 (2001).
5. Bours, V. *et al.*, *Biochemical Pharm.* **60**, 1085–1090 (2000).
6. Baldwin, A. S., *J. Clin. Invest.* **107** (1), 3–6 (2001).
7. Tran, K. *et al.*, *Mol. Cell. Biol.* **17** (9), 5386–5399 (1997).
8. Park, Y-W. *et al.*, *Anal Biochem.* **269**, 94–104 (1999).
9. Zhang, J-H. *et al.*, *J. Biomolecular Screening* **2**, 67–73 (1999).

7. Related products

FARCyte™ Fluorescence Plate Reader	VF129017
Cy™5 anti-Phosphotyrosine antibody	PA92000
Cy5 anti-Glutathione S-transferase antibody	(1 mg) PA92002
Cy5 Streptavidin	(1 mg) PA92005
Eu (TMT) anti-Phosphotyrosine antibody	(50 µg) PA92001 (1 mg) PA92007
Eu (TMT) anti-Glutathione S-transferase antibody	(50 µg) PA92003 (1 mg) PA92009
Eu (TMT) Streptavidin	(50 µg) PA92004 (1 mg) PA92010
Additional Cy5 reagents: all mono-reactive fluors	
Cy5 NHS Ester, 1 mg	PA15101
Cy5 NHS Ester, 5 mg	PA15105
Cy5 NHS Ester, 10 mg	PA15104
Cy5 NHS Ester, 25 mg	PA15106
Cy5.5 NHS Ester, 1 mg	PA15601
Cy5.5 NHS Ester, 5 mg	PA15605
Cy5.5 NHS Ester, 10 mg	PA15606
Related CyDye products	
Cy3 NHS Ester, 1 mg	PA13101
Cy3.5 NHS Ester, 1 mg	PA13601
Cy3B NHS Ester, 1 mg	PA63101
Cy7 NHS Ester, 1 mg	PA17101
Cy5Q NHS Ester, 1 mg	PA75101
Cy7Q NHS Ester, 1 mg	PA77101

Cy3 NHS Ester, 5 mg	PA13105
Cy3.5 NHS Ester, 5 mg	PA13605
Cy3B NHS Ester, 5 mg	PA63100
Cy7 NHS Ester, 5 mg	PA17105
Cy5Q NHS Ester, 5 mg	PA75100
Cy7Q NHS Ester, 5 mg	PA77100

Also available as 10 mg, 25 mg and 50 mg packs on request.

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