Proteins

TR-FRET

Custom labeling services & catalog reagents

NEW product

For Life Science Research

TR-FRET TECHNOLOGY

TR-FRET is a combination of Time Resolved Fluorescence (TRF) and Forster's Resonance Energy Transfer (FRET) technologies. This method offers the advantages of reducing background noise and providing a **higher sensitivity and reliability than the FRET method.**

In **TR-FRET** instead of a fluorescent dye, the donor molecule is a Lanthanide metal.

Competitively priced for your convenience

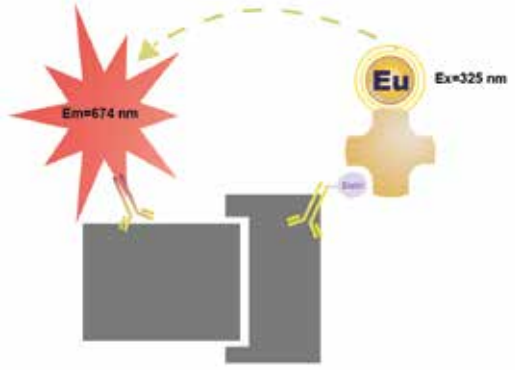


Fig 1. Scheme of TR-FRET

BENEFITS

Ideal for HTS

Homogeneous mix and read assay format

Large dynamic range

The large Stokes shift that characterizes lanthanides allows for the use of broad excitation/emission filters

High sensitivity

TR-FRET uses lanthanides with long-lived fluorescence. This gives a higher signal to noise ratio.

Low interference

The delay between excitation and emission is longer than with conventional dyes, thereby reducing fluorescent interference

APPLICATIONS

> FRET/TR-FRET substrates for protease activity detection

> Protease based drug target screening and discovery

> Protein-peptide interaction

> Protein-protein interaction

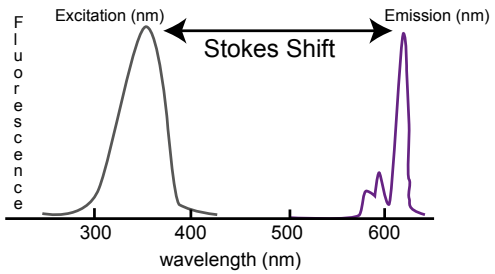
TR-FRET like FRET involves an energy transfer between two fluorophores in close proximity.

Lanthanide metals, such as Terbium (Tb) and Europium (Eu), serve as energy donors. Their **fluorescence is long-lived** and is characterized by a large Stokes shift providing a high signal-to-noise ratio due to minimal crosstalk between excitation and emission wavelengths. The delay between excitation and emission detection minimizes prompt fluorescence interference (coming from biological samples).

GOOD TO KNOW

Typically biological samples generate fluorescence (proteins, antibodies, cells or tissues) that can interfere with the assay observations and therefore decrease the assay sensitivity.

TR (time-resolved) based assay technologies eliminate the background by choosing a longer detection interval after excitation, therefore increasing the assay sensitivity.



Stokes shift is the difference between the absorption peak and the emission peak for fluorophores. Larger Stokes shift is always preferred which allows the use of broad excitation and emission filters that do not overlap, which in turn increases brightness and sensitivity. Fluorescent probes of smaller Stokes shift require filters that are very close together and do not include the entire area of the curves, thus reducing efficiency and brightness.

Fig 2. Stokes shift absorption and emission peaks for Europium.

Property	Lanthanide	Conventional
Fluorescence Lifetime	600-1000 microseconds	nanoseconds
Stokes shift	290 nm (Eu) separation of Ex/Em	<100 nm
Emission profile	Narrow (sharp peak)	Broad (shoulder peak)

CUSTOM SERVICES*

We are proud to extend our offering of TR-FRET products and service backed by our high degree of expertise in protein labeling, FRET & fluorogenic peptide substrate synthesis, and assay development. Our Europium chelate/fluorophore pairs are designed to achieve overlap of the donor emission and the acceptor absorbance spectra.

Eu is typically paired with redshifted dyes (allophycocyanine, Cy®5/CyLyte Fluor 5, or HiLyte™ Fluor 647/Alexa Fluor® 647).

Protein and Peptide - Substrate Labeling

FEATURES

- Specific Degree of substitution (DOS)
- Custom Formulation
- Custom QC available
  - SDS-PAGE
  - Mass spectrometry
  - Protein Activity

LABEL

Donor	Ex/Em	Acceptor
Europium chelate	325/620 nm	HiLyte™ Fluor 647
Europium chelate	325/620 nm	CyLyte Fluor 5

CERTIFICATES OF ANALYSIS

Protein CoA Includes

- Spectral properties
- DOS: represents the amount of fluorophore molecules conjugated to one protein molecule
- Formulation, buffer composition
- Amount and concentration
- Optimal storage conditions

Peptide CoA Includes

- Lyophilized peptide:
  - Mass Spec Analysis
  - HPLC Analysis for purity
  - Amount
- Liquid formulations also include:
  - Concentration
  - Spectral properties
  - Optimal storage conditions

Assay Design and Development

- Preparation of substrates labeled with Europium and/or HiLyte™ Fluor 647
- Validation of assay with inhibitor(s) or biological compounds
- Detailed step by step assay protocol.

*We know that confidentiality is important to you, and will treat your project and data with the utmost care and respect.

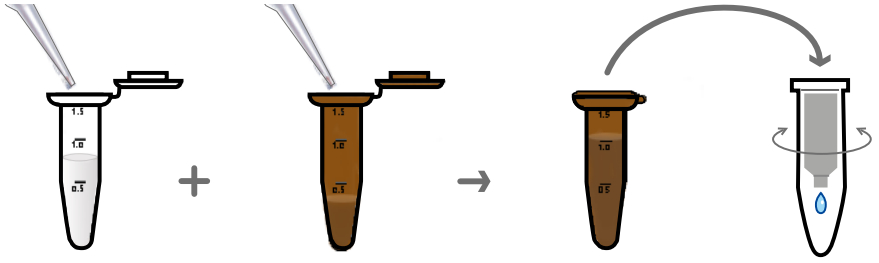
CONTACT US

Contact **biology@anaspec.com** to discuss your project

KITS AND REAGENTS

AnaTag™ Labeling Kits FOR TR-FRET

Conveniently conjugate your own TR-FRET Protein reagents with our amine reactive Europium chelate and HiLyte™ Fluor 647 labeling kits.



Step 1. Add reaction buffer to protein (ie., IgG)

Step 2. To dye, add solvent and incubate as per protocol

Step 3. Mix dye with protein, incubate as per protocol

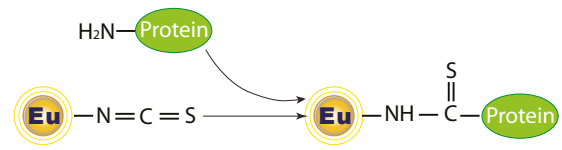
Step 4. Purify using a spin column (microscale kit)

BENEFITS

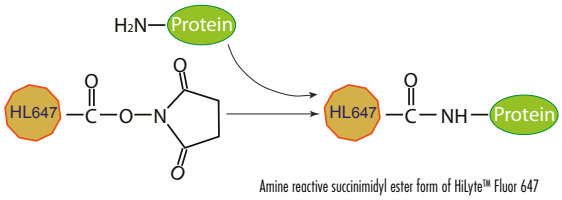
> Fast labeling
> Stable dye-protein conjugates
> All essential components for conjugation and purification included.

ORDERING INFORMATION

Label	Kit Description	Ex/Em	Cat #
Europium chelate	AnaTag™ Europium Protein Labeling Kit	325/620 nm	AS-72246
HiLyte™ Fluor 647	AnaTag™ HiLyte™ Fluor 647 Protein Labeling Kit	649/674 nm	AS-72049
	AnaTag™ HiLyte™ Fluor 647 Microscale Protein Labeling Kit	649/674 nm	AS-72050
Europium chelate & HiLyte™ Fluor 647	AnaTag™ Protein labeling kit for TR-FRET	Eu: 325/620 nm HL 647: 649/674 nm	AS-72247



Amine reactive isothiocyanate reactive group on Europium



Amine reactive succinimidyl ester form of HiLyte™ Fluor 647

Streptavidin FOR TR-FRET

Streptavidin is a non-glycosylated, tetrameric protein, with each subunit able to bind a single molecule of biotin. Streptavidin-biotin bond is the strongest known non-covalent interaction with Kd ~10^{-15} M. Because streptavidin lacks any carbohydrate modification and has a near-neutral pI, it has the advantage of much lower nonspecific binding than avidin. Streptavidin is broadly used in various applications.

ORDERING INFORMATION

Detection Reagent	Ex/Em	Cat #
Streptavidin, Europium labeled	325/620 nm	AS-72252
Streptavidin, HiLyte™ Fluor 647 conjugated	649/674 nm	AS-60667

Suitable to use with any biotinylated antibody

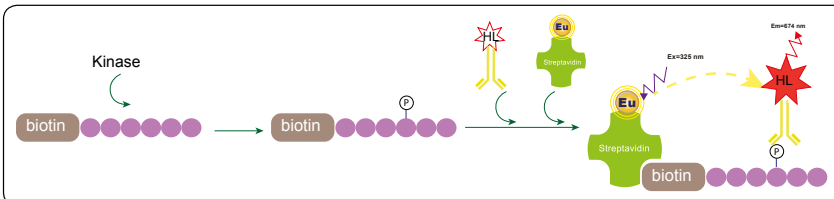


Fig 3. TR-FRET between Europium and HiLyte™ Fluor 647 for the detection of kinase activity.

In this scheme, a biotinylated peptide is phosphorylated due to the presence of a kinase. The phosphorylation site is then recognized by a HiLyte™ Fluor 647 labeled anti-phospho antibody, while Europium labeled streptavidin binds to biotin, resulting in TR-FRET.

Antibodies FOR TR-FRET

ORDERING INFORMATION

HiLyte™ Fluor 647 labeled Antibody & Protein A	Ex/Em	Cat #
Mouse Monoclonal anti-His Tag IgG, HiLyte™ Fluor 647 labeled	649/674 nm	AS-61250-H647
Mouse Monoclonal anti-GST Tag IgG, HiLyte™ Fluor 647 labeled	649/674 nm	AS-29531-H647
Rabbit anti-His Tag IgG, HiLyte™ Fluor 647 labeled	649/674 nm	AS-29673-H647
Rabbit anti-Actin (alpha Smooth muscle specific), HiLyte™ Fluor 647 labeled	649/674 nm	AS-29553-H647
Rabbit anti-Mouse IgG (H+L), HiLyte™ Fluor 647 labeled	649/674 nm	AS-28164-05-H647
Rabbit anti-Goat IgG (H+L), HiLyte™ Fluor 647 labeled	649/674 nm	AS-28168-05-H647
Goat anti-Rabbit IgG (H+L), highly cross-adsorbed, HiLyte™ Fluor 647 labeled	649/674 nm	AS-61056-05-H647
Goat anti-Mouse IgG (H+L), highly cross-adsorbed, HiLyte™ Fluor 647 labeled	649/674 nm	AS-61057-05-H647
Protein A - HiLyte™ Fluor 647 Conjugate	649/674 nm	AS-72237

ORDERING INFORMATION

Europium labeled Antibody	Ex/Em	Cat #
Mouse Monoclonal anti-His tag IgG, Eu labeled	325/620 nm	AS-72251
Mouse Monoclonal anti-GST tag IgG, Eu labeled	325/620 nm	AS-72250
Goat anti-Rabbit IgG (H+L), highly cross-adsorbed, Eu labeled	325/620 nm	AS-72249
Goat anti-Mouse IgG (H+L), highly cross-adsorbed, Eu labeled	325/620 nm	AS-72248

HOW TO ORDER

WWW.ANASPEC.COM

CATALOG PRODUCTS

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1. Register or login to **www.anaspec.com**
2. Select your product(s)
3. Add them to your cart

EMAIL

1. Send order request to **orders@anaspec.com**
2. Include product name(s), catalog number(s) & quantity(ies)

CUSTOM SERVICES

Contact **biology@anaspec.com** to discuss your project

LICENSE STATEMENTS

HiLyte™ Fluor Dyes

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Alexa Fluor® dyes

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