TECHNICAL DATA SHEET

THUNDER™ TR-FRET Acceptor-labeled Streptavidin

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CATALOG NUMBERS FRSA-1000 (1000 points)
FRSA-5000 (5000 points)
FRSA-20000 (20000 points)

Store at -20°C.
For research use only.
Not for use in diagnostic procedures.

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PRODUCT DESCRIPTION

THUNDER™ TR-FRET Toolbox reagents are designed for setting up homogeneous (no-wash) time-resolved Förster resonance energy transfer (TR-FRET) assays using Acceptor chelate (Eu) as the donor fluorophore and a small far-red dye (FR) as the acceptor fluorophore. The Acceptor-labeled streptavidin can be used for capturing biotinylated molecules in THUNDER™ TR-FRET assays.

PRODUCT INFORMATION

Protein	Streptavidin is a tetrameric biotin binding protein that is isolated from *Streptomyces avidinii*.
Volume	400 µL (1000 points); 2 mL (5000 points); 8 mL (20000 points);
Molecular Weight	60,000
Storage Conditions	Store at -20°C protected from light. Repeated freezing and thawing should be avoided.
Stability	This product is stable for at least 22 months from the manufacturing date when stored in its original packaging and at the recommended storage conditions.

RECOMMENDED ASSAY CONDITIONS

Reagent Preparation

- The number of assay points is based on an assay volume of 20 µL in low-volume 384-well assay plates using the reagent at the recommended concentration.
- We cannot guarantee the performance of the product outside the conditions detailed in this Assay Protocol.
- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube.
- Bring the reagent to room temperature (RT) prior to running the assay.
- Centrifuge the tube before use to improve recovery of content (2000x g, 10-15 sec).
- Use ultrapure water (Milli-Q® grade water; 18 MΩ cm) to prepare the Assay Buffer and TR-FRET Detection Buffer.
- The toolbox reagents are designed to work with the THUNDER 1X TR-FRET Detection Buffer for maximum performance. Other Detection Buffers might work but have not been tested.

STEP 1 PREPARATION OF THE **1X TR-FRET DETECTION BUFFER**

A. Dilute 10-fold the 10X TR-FRET Detection Buffer (catalog No. TRFRET-DB) in ultrapure water.
B. For example, add 100 µL of 10X Detection Buffer to 900 µL of ultrapure water.
C. The unused 1X Detection Buffer may be stored at 4°C for 2 days.

STEP 2 PREPARATION OF THE **4X WORKING SOLUTION OF EUROPIUM-LABELED REAGENT**

A. Dilute 50-fold the stock solution in 1X TR-FRET Detection Buffer.
B. For example, add 10 µL of stock solution to 490 µL of 1X TR-FRET Detection Buffer. Mix gently.
C. The unused 4X Working Solution can be stored at 4°C for 2 days.

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RECOMMENDED ASSAY CONDITIONS (CONTINUED)

STEP 3 PREPARATION OF THE **4X WORKING SOLUTION OF FR-STREPTAVIDIN**

A. Dilute 12.5-fold the stock solution in 1X TR-FRET Detection Buffer.
B. For example, add 40 µL of stock solution to 460 µL of 1X TR-FRET Detection Buffer. Mix gently.
C. The unused 4X Working Solution can be stored at 4°C for 2 days.

NOTE: *We recommend using the Acceptor-labeled and Acceptor-labeled reagents at the 1X final concentration. However, the optimal concentration of labeled reagents will be dependent on the assay conditions. The optimal concentrations can be determined by testing each reagent at 0.5X, 1X, 2X, and 4X final concentrations.*

Standard Assay Protocol

Run the assay in a white low-volume 384-well microplate as follows:

- Conduct the reaction step in Assay Reaction Buffer in a 10-µL volume.
- Add 5 µL of 4X Eu-labeled streptavidin (1X final concentration) in 1X TR-FRET Detection Buffer.
- Add 5 µL of 4X FR-labeled reagent (1X final concentration) in 1X TR-FRET Detection Buffer.
- Cover the plate with sealing film and incubate for desired time at RT.
- Remove the sealing film and read TR-FRET signal (excitation at 320 or 340 nm; emission at both 615 nm and 665 nm).

VALIDATION DATA

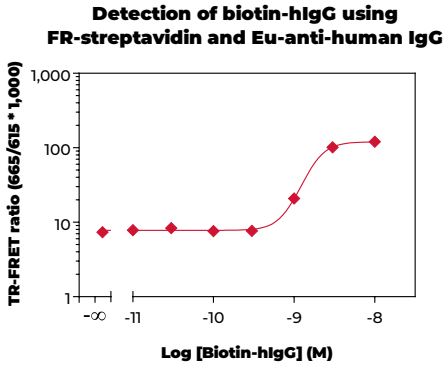

Figure 1 Titration of biotinylated human IgG using FR-labeled streptavidin, Eu-labeled anti-human IgG and THUNDER 1X TR-FRET Detection buffer. The plate was read on an EnVision® plate reader (flash lamp excitation) after 1 hour of incubation at room temperature.

RECOMMENDATIONS

- Assay Reaction Buffers: THUNDER™ TR-FRET technology works with a wide range of buffers and additives, and is highly tolerant towards many commonly used chemicals, including detergents, BSA, and DMSO. We recommend starting with a Tris- or HEPES-based buffer supplemented with salt, detergent, and BSA as needed, with a pH maintained between 5.5 and 8.5, and then further optimize as necessary. A typical Assay Buffer is 50 mM HEPES pH 7.4, 100 mM NaCl, 0.1% Triton X-100, and 0.1% BSA. Avoid using phosphate buffers in your assay.
- Assay plates: we recommend using white low-volume 384-well microplates.
- When loading reagents in the assay microplate, change tips between each reagent addition.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with an adhesive sealing film to reduce evaporation during incubation. You must remove the film before reading.
- The TR-FRET signal is detected using a reader equipped with the TR-FRET option. Use an excitation wavelength of 320 or 340 nm to excite the Acceptor chelate. It is highly recommended to read TR-FRET assays in dual emission mode, detecting both the emission from the Acceptor donor at 615 or 620 nm, and the acceptor fluorophore at 665 nm.
- Data analysis: you can use the signal at 665 nm or you can normalize data against the Acceptor signal at 615 nm using the following formula: [(665 nm/615 nm) x 1,000].
- NOTE: TR-FRET signal will vary between instruments and with incubation time.

FOR MORE INFORMATION ABOUT THUNDER™ TR-FRET, ASSAY SET UP, AND COMPATIBLE READERS, PLEASE VISIT bioauxilium.com