TECHNICAL DATA SHEET

THUNDER™ Reader Control Assay Kit

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CATALOG NUMBER KIT-CTRL-100 (100 tests)

Store at -80°C.
For research use only.
Not for use in diagnostic procedures.

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PRODUCT DESCRIPTION

This assay kit is designed to quickly assess the suitability of TR-FRET compatible microplate readers to perform Europium-based TR-FRET measurements. It can be used to set up the instrument, to verify and optimize its measurement settings, to assess and validate its performance, and for the comparison of different readers. The kit uses a simple and rapid (10 minutes) assay based on the homogeneous (no-wash) THUNDER™ TR-FRET technology.

TR-FRET ASSAY PRINCIPLE

The THUNDER™ Reader Control assay kit is a homogeneous (no-wash) time-resolved Förster resonance energy transfer (TR-FRET) assay (Figure 1). The assay workflow consists of two reagent additions (Figure 2). One binding partner is labeled with a Europium chelate donor (Europium conjugate) and the second with the far-red acceptor fluorophore (Acceptor conjugate). The binding of the two labeled partners takes place in solution and brings the two dyes into close proximity. Excitation of the donor Europium chelate molecules with a flash lamp (320 or 340 nm) or a laser (337 nm) triggers a FRET from the donor to the acceptor molecules, which in turn emit a TR-FRET signal at 665 nm. Residual energy from the Eu chelate generates light at 615 nm.

Figure 2 THUNDER™ Reader Control assay workflow

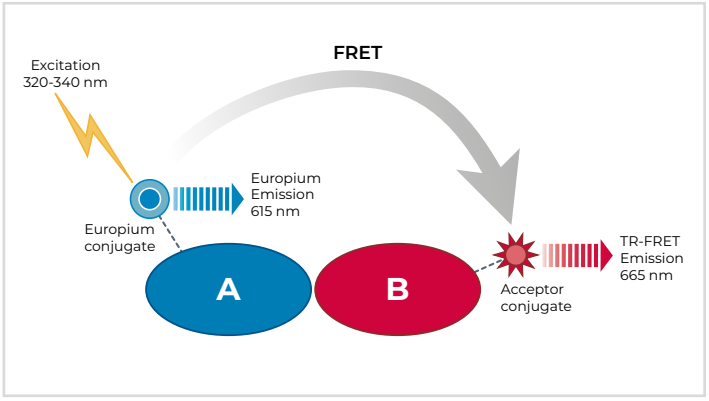

Figure 1 Schematic representation of the Reader Control assay principle.

KIT COMPONENTS	100 points*
Europium conjugate (50X)	20 µL (1 clear tube, red cap)
Acceptor conjugate (50X)	20 µL (1 brown tube, blue cap)
Control Buffer (10X)	500 µL (1 tube, yellow cap)

* The number of assay points is based on an assay volume of 20 µL in low-volume 384-well assay plates using the kit components at the recommended concentrations.

ADDITIONAL MATERIALS REQUIRED	Recommended source	Catalog No.
Ultrapure laboratory grade water	Many options available	NA
Low-volume 384-well microplate, white	PerkinElmer Greiner Corning	6007290 784075 4513
Half-area 96-well microplate, white	PerkinElmer Greiner Corning	6052480 675075 3693
Single-channel pipette	Many options available	NA
Adhesive sealing film for plates	Many options available	NA
A plate reader equipped with the TR-FRET option	Many options available	NA

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THUNDER™ ASSAY PROTOCOL

Upon receipt, store kit in the dark at -80°C.

This protocol must be read in its entirety prior to beginning the assay.

- We cannot guarantee the performance of the product outside the conditions detailed in this Assay Protocol.
- Bring all reagents to room temperature prior to running the assay.
- Centrifuge all tubes before use to improve recovery of content (2000x g, 10-15 sec).
- Use ultrapure water (Milli-Q® grade water; 18 MΩ cm) to dilute Assay Buffer.
- It is recommended to test all standards in triplicate and samples at least in duplicate.

REAGENT PREPARATION

- The instructions described below are for testing the entire number of assay points in each kit. Adjust volumes accordingly for testing of fewer assay points.
- Prepare only as much reagent as is needed on the day of the experiment.

STEP 1 PREPARATION OF 1X CONTROL BUFFER

- The thawed 10X Control Buffer can be stored at 4°C for 1-2 weeks. For longer periods of time, buffer should be stored at -80°C.
- The unused 1X Control Buffer may be stored at 4°C for 1 day.

A. Mix end-over-end the 10X Control Buffer before use.

B. Dilute 10-fold the 10X Control Buffer in ultrapure water. For example, add 500 µL of 10X Control Buffer to 4.5 mL of ultrapure water.

STEP 2 PREPARATION OF 1X WORKING SOLUTION OF EUROPIUM CONJUGATE

Dilute 50-fold the 50X Europium Conjugate stock solution in 1X Control Buffer. For example, add 20 µL of stock solution to 980 µL of 1X Control Buffer. Mix gently.

STEP 3 PREPARATION OF 1X WORKING SOLUTION OF ACCEPTOR CONJUGATE

Dilute 50-fold the 50X Europium Conjugate stock solution in 1X Control Buffer. For example, add 20 µL of stock solution to 980 µL of 1X Control Buffer. Mix gently.

ASSAY PROCEDURE

- We recommend plating a minimum of 3 replicates of each reagent.
- When loading reagents in the low-volume 384-well microplate, change tips between each set of reagents.
- Run the assay in a white low-volume 384-well microplate as follows:

For HIGH CONTROL wells:

A. Add 10 µL of 1X Europium conjugate
B. Add 10 µL of 1X Acceptor-conjugate

For Europium BLANK wells:

C. Add 10 µL of 1X Europium conjugate
D. Add 10 µL of 1X Control Buffer

For Acceptor BLANK wells:

E. Add 10 µL of 1X Acceptor conjugate
F. Add 10 µL of 1X Control Buffer

For Buffer BLANK wells:

G. Add 20 µL of 1X Control Buffer

H. Cover the plate with sealing film and incubate for 10 minutes at room temperature.

I. Gently remove the sealing film and read TR-FRET signal.

SUMMARY OF PIPETTING PROTOCOL

	HIGH CONTROL wells	Europium BLANK wells	Acceptor BLANK wells	Buffer BLANK wells
Europium conjugate	10 µL	10 µL		
Acceptor conjugate	10 µL		10 µL	
1X Control Buffer		10 µL	10 µL	20 µL
Total assay volume	20 µL	20 µL	20 µL	20 µL

NOTE *The same plate can be read several times without any negative effect on the assay performance.*

TR-FRET PLATE READER SETTINGS

Read the assay plate at two wavelengths, detecting both the emission from the Europium chelate donor fluorophore at 615 nm (or 620 nm), and the acceptor fluorophore at 665 nm. The following instrument settings are provided as guidelines.

	TR-FRET Compatible Plate Reader*	
Parameter	**Flash lamp excitation**	**Laser excitation**
Excitation filter	320 nm (or 340 nm)	Not applicable
Emission filter	615 nm (or 620 nm)	615 nm (or 620 nm)
Delay time	90 µs	50 µs
Flash energy level	100% or High	100%
Number of flashes	100-200	20
Window (integration time)	300 µs	100 µs

* These settings are provided as guidelines only. Settings should be optimized for each reader.

SIGNAL DETECTION AND DATA ANALYSIS

The test consists of two measurements:

- The first one evaluates the Signal-to-Blank (S/B) ratio of the Europium donor channel (emission at 615 or 620 nm).
- The second one evaluates the S/B ratio of the acceptor fluorophore channel (emission at 665 nm).
- For each well: record the donor emission values (signal at 615 or 620 nm) and the acceptor emission values (signal at 665 nm). Calculate the average values (Mean) and percent coefficient of variation (CV%).
- **S/B ratio of the Europium donor channel.** Calculate the S/B ratio obtained with the Europium conjugate alone: divide the average signal at 615 nm obtained with the Europium conjugate (Europium BLANK) by the average signal at 615 nm obtained with the Control Buffer alone (Buffer BLANK).

	665 nm		615 nm	
	Mean	**CV%**	**Mean**	**CV%**
Europium BLANK				
Buffer BLANK				
S/B ratio				
Acceptor BLANK				
HIGH control				

- For the HIGH CONTROL and Europium BLANK wells: calculate the TR-FRET ratio for each well using the following equation: [(665 nm/615 nm) x 1,000].
- **S/B ratio of the Acceptor fluorophore channel.** Calculate the S/B ratio obtained with the Europium/Acceptor conjugate Mix: divide the average TR FRET ratio obtained with the Europium/Acceptor conjugate Mix (HIGH CONTROL) by the average TR-FRET ratio obtained with the Europium conjugate alone (Europium BLANK).

	TR-FRET ratio	
	Mean	**CV%**
HIGH control		
Europium BLANK		
S/B ratio		

- Keep all data for reference using the datasheet provided in the Appendix.

APPENDIX

Test datasheet

Microplate reader (brand and model):	
Serial number:	
Date of test:	
Lot number of THUNDER™ Reader Control TR-FRET Kit:	
Microplate used:	
Instrument settings:	

Parameter	Setting
Light source	
Mirror	
Excitation filter	
Emission filter 1	
Emission filter 2	

Parameter	Setting
Gain value	
Delay time	
Flash energy level	
Number of flashes	
Integration time	

	665 nm		615 nm	
	Mean	CV%	Mean	CV%
Europium BLANK				
Buffer BLANK				
S/B ratio				
Acceptor BLANK				
HIGH control				

	TR-FRET ratio	
	Mean	CV%
HIGH control		
Europium BLANK		
S/B ratio		