Monoclonal Anti-VHH Antibody FA-Labeled (for TR-FRET)

Catalog Number: FRT-TA12

Pack Size: 1000 tests & 5000 tests & 20000 tests

Assay Volume: 20 μL

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

PRODUCT OVERVIEW

The Monoclonal Anti-VHH Antibody FA-Labeled is a universal Acceptor designed to bind Humanized and Alpaca VHHs in a TR-FRET Assay. This product is generated by covalent conjugation of a fluorescent dye (FA) to a high-affinity Monoclonal Anti-Camelid VHH Antibody, Rabbit IgG. This antibody recognizes Humanized and Alpaca VHHs.

In TR-FRET assays, this Acceptor can be used in combination with a suitable lanthanide chelate-labeled Donor, such as Europium chelate. TR-FRET signal generation requires that both the Donor and Acceptor bind to their respective target molecules, thereby bringing the Donor and Acceptor into close proximity. Upon donor excitation with light of a specific wavelength (337 nm), in addition to Donor emission (620 nm), non-radiative transfer of energy occurs between Donor and Acceptor, resulting in Acceptor emission (665 nm).

SPECIFICATION

TABLE 1. SPECIFICATIONS

Content Item	Size (1000 tests)	Size (5000 tests)	Size (20000 tests)
Amount	1 vial	1 vial	1 vial
Formulation	Lyophilized from 0.22 μm filtered solution in PBS, pH7.4 with trehalose as protectant.		
Physical Appearance	Powder	Powder	Powder

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MATERIALS REQUIRED BUT NOT PROVIDED

TABLE 2. MATERIALS REQUIRED BUT NOT PROVIDED

Items	Specifications	Recommendation
Europium or other suitable chelate Toolbox	As the Donor	ACRO, Please click to view https://www.acrobiosystems.cn/category/solutions/tr-fret
TR-FRET Sample Dilution Buffer, pH7.4	For sample dilution	ACRO (Cat. No. DB-04)
TR-FRET Detection Buffer, pH7.4	For Donor (Europium chelate) and Acceptor dilution in pH7.4 reaction system	ACRO (Cat. No. DB-05)
TR-FRET Detection Buffer, for pH6.0 reaction system	For Donor (Europium chelate) and Acceptor dilution in pH6.0 reaction system	ACRO (Cat. No. DB-06)
TR-FRET Detection Buffer, for pH5.5 reaction system	For Donor (Europium chelate) and Acceptor dilution in pH5.5 reaction system	ACRO (Cat. No. DB-07)
TR-FRET Detection Buffer, for pH5.0 reaction system	For Donor (Europium chelate) and Acceptor dilution in pH5.0 reaction system	ACRO (Cat. No. DB-08)
TR-FRET Terbium Detection Buffer, pH7.4	For Donor (Terbium chelate) and Acceptor dilution in pH7.4 reaction system	ACRO (Cat. No. DB-11)
Single channel and multi-channel pipettes	Must be calibrated pipettes, with 10 µL, 200 µL and 1000 µL precision	Different pipettes have different levels of precision. Please choose pipettes with appropriate precision.
Pipette tips	Low adsorption pipette tips, all tips need to fit the pipettes	-
96 or 384-well white plate	Non-transparent 96- or 384-well low-volume white plates typically provide the lowest background signal.	For example, 384-well white plate (iSTAR, Cat. No. GT247.008)
Microplate shaker	For plate shaking	-
EP tubes	For dilution of samples	-
Microplate reader	Plate reader capable of measuring signals at 665 nm/620 nm in TR-FRET mode	For example, BMG LABTECH CLARIOstar® Plus; TECAN Spark®, Infinite® F Nano+, Infinite® F Plex
Timer	-	-
Deionized or distilled water	For reconstitution	-

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STORAGE AND VALIDITY INSTRUCTIONS

For long term storage, the product should be stored in lyophilized state at -20°C or lower.

Please avoid repeated freeze-thaw cycles.

This product is stable after storage at:

- -20°C to -70°C for 12 months in lyophilized state from date of receipt.
- -70°C for up to 3 months after reconstitution, protected from light and stored under sterile conditions.

ASSAY FORMAT

When using our three available pack sizes (1000 tests, 5000 tests and 20000 tests), we recommend a total reaction volume of 20 μL for each experiment.

Please select the appropriate Donor and experimental components based on the experimental objectives.

Please Note:

1. ***Precursors of Donor and Acceptor must be different. For example, if you select the Monoclonal Anti-Mouse IgG Antibody FA-Labeled (Cat. No. FRT-TA03) as the Acceptor, you must not choose the Monoclonal Anti-Mouse IgG Antibody Europium chelate (Cat. No. FRT-TD03) as the Donor, as both would bind the same target components.***
2. ***Donor and Acceptor must not react with each other directly.***
3. ***The Donor and Acceptor must only bind their designated target components and should not react with any other components. Otherwise, non-specific interactions may occur, which could lead to experimental failure.***

TABLE 3. ASSAY FORMAT

Assay Format	Volume
Other assay components	10 μL
Donor conjugate	5 μL
Acceptor conjugate	5 μL

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ASSAY PROCEDURE

1. Reagent Preparation

Bring all reagents and samples to room temperature (20℃-25℃) before use.

2. Material Preparation

Prepare materials and tools for your experiment, such as pipettes and tips, white 96 or 384-well plate, EP tubes, Sample Dilution Buffer and Detection Buffer, etc. The details could refer to the table "***MATERIALS REQUIRED BUT NOT PROVIDED***".

3. Switch on the microplate reader and set the parameters according to the experimental requirements.

4. Stock Solution Preparation

Reconstitute all lyophilized components into stock solutions with the volumes of water as described in their accompanying instructions or Certificate of Analysis (CoA). Allow the solutions to solubilize for 15-30 minutes at room temperature with occasional gentle mixing by inverting the tube 2-3 times. **Avoid vigorous shaking or vortexing**. The reconstituted stock solutions should be stored at -70℃. It is not recommended to freeze-thaw the reconstituted stock solution more than 3 times.

It is strongly recommended to reconstitute the lyophilized Monoclonal Anti-VHH Antibody FA-Labeled with sterile deionized water as indicated in Table 4 below.

Note: both Donor and Acceptor stock solutions should be protected from light.

TABLE 4. RECONSTITUTION METHODS

Monoclonal Anti-VHH Antibody FA-Labeled (for TR-FRET)	Reconstitution Buffer	Reconstitution Volume
1000 tests	Sterile deionized water	25 µL
5000 tests	Sterile deionized water	125 µL
20000 tests	Sterile deionized water	500 µL

5. Buffer Preparation

- The relevant buffers can be found in the table "***MATERIALS REQUIRED BUT NOT PROVIDED***", which have been optimized for maximum performance.

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- You can select a suitable Detection Buffer according to the pH of the reaction system. The Detection Buffer is designed for diluting the Donor and the Acceptor conjugates. The TR-FRET Sample Dilution Buffer, pH7.4 (ACRO, Cat.No.DB-04) is recommended for dilution and preparation of other assay components. The buffers for Donor and the Acceptor conjugates are the same.
- If you wish to use a homemade buffer solution, you can prepare it according to the needs of your experimental system. A buffer such as PBS (Phosphate Buffered Saline) or HEPES containing 0.1%-0.5% BSA and 0.01%-0.05% Tween-20 with the pH maintained between 5.5 and 8.5 is recommended. Avoid SDS due to its denaturing effect on FA fluorescence. Using Europium conjugates as Donor requires a final KF (Potassium Fluoride) concentration between 100 mM and 400 mM. The use of Terbium conjugates as Donor does not require KF.

6. Working Solution Preparation

You should choose suitable concentrations to dilute each component and determine the sample loading volumes according to your experimental requirements. Dilute the Monoclonal Anti-VHH Antibody FA-Labeled stock solution 200 times with TR-FRET Detection Buffer to prepare the Acceptor working solution. (e.g. add 5 µL of stock solution to 995 µL of TR-FRET Detection Buffer.). This working solution should be prepared immediately before use and should not be stored.

7. Experimental System Configuration

Based on your experimental objectives, design an appropriate experimental protocol and configure the assay system.

8. Data Recording

Use the TR-FRET module of a microplate reader to measure the fluorescence signal at 665 nm and 620 nm (the excitation wavelength is 337 nm).

9. Calculate Ratio

For each individual well, calculate the ratio of the acceptor (665 nm) and donor (620 nm) emission signals using the formula below:

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4.$$

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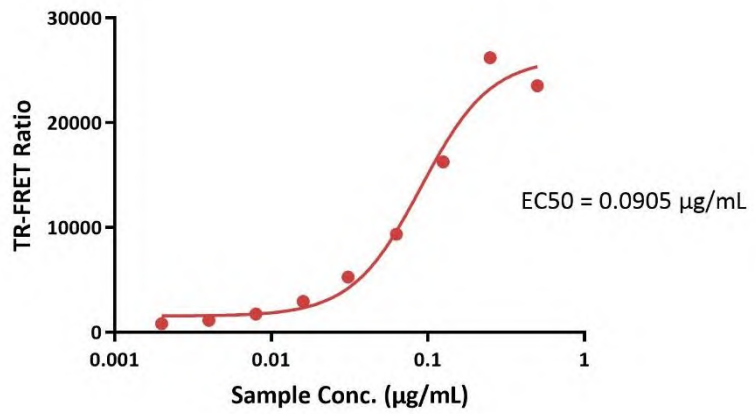
TYPICAL DATA

For each experiment, a standard curve should be established for every microplate, and the specific Ratio values may vary depending on the laboratories, operator, and equipment. Different microplate readers and different gain settings may produce varying fluorescence signals. Please adjust the parameters according to the user manual. Reduce the gain value if the signal is too high. The following data were obtained using the BMG LABTECH PHERAstar® and are provided for reference only.

Monoclonal Anti-His Tag Antibody Europium chelate (Cat. No. FRT-TD05) paired with Monoclonal Anti-VHH Antibody FA-Labeled (Cat. No. FRT-TA12)

Sample Conc. (μg/mL)	Ratio	SD	CV (%)
0.5	23535	517	2.20
0.25	26208	379	1.45
0.125	16274	220	1.35
0.063	9354	46	0.49
0.031	5269	29	0.56
0.016	2946	34	1.16
0.008	1745	8	0.47
0.004	1148	17	1.44
0.002	814	13	1.66
0	508	6	1.22



In this TR-FRET assay, Monoclonal Anti-His Tag Antibody Europium chelate (Cat. No. FRT-TD05) is used as the Donor and Monoclonal Anti-VHH Antibody FA-Labeled (Cat. No. FRT-TA12) is used as the Acceptor. Human 4-1BB, His Tag (Cat. No. 41B-H52Hc), at 12.5 ng/well, can bind to the Monoclonal Anti 4-1BB VHH Antibody with a linear detection range of 0.5-0.002 μg/mL (QC tested).

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