DB-04-EN.01

TR-FRET Sample Dilution Buffer, pH7.4

Catalog Number：DB-04

Pack Size: 50 mL

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

PRODUCT OVERVIEW

This TR-FRET Sample Dilution Buffer is designed for sample dilution in TR-FRET Assay, and this buffer is particularly well suited when Europium-chelate is involved in the TR-FRET Assay.

SPECIFICATION

Table 1. Specifications

Content Item	Specifications
Composition	1×PBS (10 mM PBS: 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 136 mM NaCl, 2.6mM KCl), with 0.5%BSA, 0.05% Tween-20 and 0.05% ProClin™300, pH 7.2-7.4
Sterilization Treatment	The Buffer is steam sterilized at high temperature and filtered through 0.2 μm filter membrane
Physical Appearance	Liquid
Unit Size	50mL
Storage	2-8℃
Technology	TR-FRET
Intended Use	For sample dilution

STORAGE AND VALIDITY INSTRUCTIONS

1. The buffer should be stored at 2℃-8℃ upon receiving.

2. Find the expiration date on the outside packaging and do not use the buffer past its expiration date.

RECOMMENDED PROTOCOL

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

2. Materials Preparation: Prepare materials and tools for your experiment, such as pipettors and tips, white 96/384-well plate, EP tubes, Microplate reader with TR-FRET module which can detect signals at 665 nm/620 nm, and Microporous plate shaker, etc.

3. Turn on the microplate reader and set the parameters according to the requirements of your experiment.

4. Stock Solution Preparation: Reconstitute the provided lyophilized Donor and Acceptor materials to stock solutions with corresponding volume of water according to the instructions or Certificate of Analysis (COA). Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vertexing. The reconstituted stock solutions should be stored at -70℃. It is recommended not to freeze-thaw more than 2 times.

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5. **Sample dilution:** Dilution the samples and standard appropriately with **Sample Dilution Buffer** (DB-04). If this Sample Dilution Buffer is used with the relevant kit, dilute the standard according to the kit's instructions.

6. **Add samples and standards:** Add 10 μL of sample and standard solution to each well according to your plate setup.

7. **Add Donor:** Dilute **Donor stock solution** appropriately with desired pH of **Detection Buffer** to make **Donor working solution**. The working solution should be prepared immediately before use and should not be stored. Add 5 μL of Donor working solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for 0.5 hours on orbital shaker at 400-600 rpm to make sure the samples and donor can react adequately.

8. **Add Acceptor:** Dilute **Acceptor** stock solution appropriately with desired pH of **Detection Buffer** to make **Acceptor working solution**. The working solution should be prepared immediately before use and should not be stored. Add 5 μL of **Acceptor working solution** to each well. Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for 0.5 hours on orbital shaker at 400-600 rpm.

9. **Data Recording:** Use the TR-FRET module of a microplate reader to read the fluorescence signal at 665 nm and 620 nm.

10. **Calculate Ratio:** Calculate Ratio based on the formula Ratio = $\frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$.

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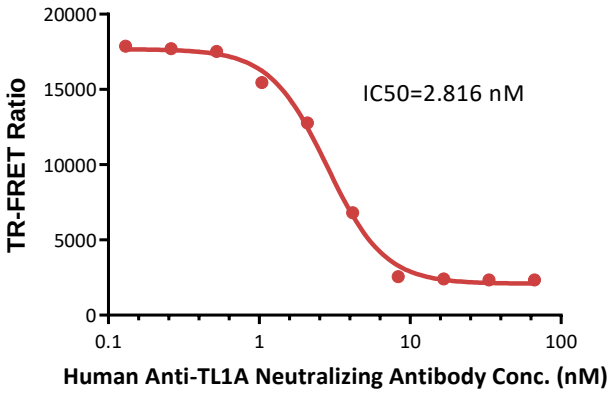
TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific Ratio value may vary depending on different laboratories, testers, or equipment. Different microplate reader and different gain value may give different fluorescence signal. Please adjust parameters according to the equipment manual. Reduce the gain value when the signal is too high. The following data is from the BMG Labtech CLARIOstar Plus. This following data is for reference only.

Sample Dilution Buffer (DB-04) used in TR-FRET kit (FRT-02):



Anti-TL1A Neutralizing Antibody		Signal 665 nm	Signal 620 nm	Ratio
Conc. (μg/mL)	Conc. (nM)			
10	66.6667	12703	54227	2343
5	33.3334	12621	53802	2346
2.5	16.6667	12263	51081	2401
1.25	8.3333	12661	49607	2552
0.625	4.1667	30277	44462	6810
0.3125	2.0833	55048	43078	12779
0.15625	1.0417	65675	42464	15466
0.078125	0.5208	68969	39344	17530
0.0390625	0.2604	74806	42242	17709
0.01953125	0.1302	73479	41091	17882
0	0	76147	41034	18557

Inhibition of Europium-chelate labeled human TL1A: FA labeled human DR3 binding by Human Anti-TL1A Neutralizing Antibody

Premix serial dilutions of Human Anti-TL1A Neutralizing Antibody (1:2 serial dilution, from 10 μg/mL to 0.01953125 μg/mL (66.6667-0.1302 nM)) and Human TL1A Protein Europium-chelate and incubate at room temperature (20℃-25℃) for 0.5 hours. Then add FA Labeled Human DR3 Protein and incubate at room temperature (20℃-25℃) for 0.5 hours. Detection was performed with IC50 of 2.816nM. The assay was performed according to the above-described Datasheet (QC tested).

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