TR-FRET Detection Buffer

Catalog Number	Description	Pack Size
DB-05	TR-FRET Detection Buffer, pH7.4	50 mL
DB-06	TR-FRET Detection Buffer, pH6.0	50 mL
DB-07	TR-FRET Detection Buffer, pH5.5	50 mL
DB-08	TR-FRET Detection Buffer, pH5.0	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 series of TR-FRET Detection Buffer are designed for Donor and Acceptor dilution in TR-FRET Assay, and is particularly well suited when Europium-chelate is involved in the TR-FRET Assay.

This assay buffer series can be used with AcroBiosystemsTM TR-FRET related kits

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, 2.6 mM KCl), with 0.2 M KF, 0.05% Tween-20 and 0.05% ProClin™300, pH 5.0-7.4
Sterilization Treatment	The buffer is steam sterilized at high temperature and filtered through 0.2 μm filter membrane
Physical Appearance	Liquid
Unit Size	50 mL
Storage	2-8℃
Technology	TR-FRET
Intended Use	For Donor and Acceptor 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. If this series of TR-FRET Detection Buffer are used with the relevant kit, dilute the Donor and Acceptor according to the kit's instructions.

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

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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.

5. **Sample and standard dilution:** Dilution the samples and standard appropriately with **Sample Dilution Buffer** (DB-04). For example, when use the **Sample Dilution Buffer** in Human FcRn binding Kit (TR-FRET) (FRT-01), 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 665nm and 620nm.

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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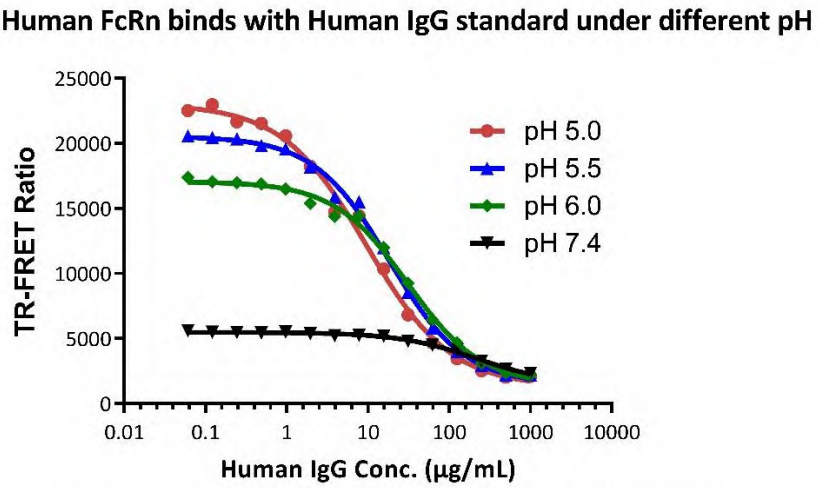
TYPICAL DATA

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.

Detection Buffer used in TR-FRET kit (FRT-01):



	pH 5.0	pH 5.5	pH 6.0	pH 7.4
IC50 (μg/mL)	9.62	18.28	31.10	221.30

The Human FcRn binding Kit (TR-FRET) has been used to detect the binding activity between Human FcRn and Human IgG standard under different pH. The IC50 shows a significant increase with increasing pH, which corelates with a decreased binding between Human FcRn and Human IgG standard.

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