Human TL1A-DR3 inhibition kit (TR-FRET)

Pack Size: 100 tests & 500 tests

Catalog Number： FRT-02

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

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

INTENDED USE

This kit is developed for screening for inhibitors of human TL1A binding to human DR3.

It is intended for research use only (RUO).

BACKGROUND

TNF-like cytokine 1A (TL1A) and its receptors, death receptor 3 (DR3) and decoy receptor 3 (DcR3) are members of the TNF and TNF receptor superfamilies of proteins, respectively. Binding of APC-derived TL1A to lymphocytic DR3 provides co-stimulatory signals for activated lymphocytes. DR3 signaling affects not only the proliferative activity of and cytokine production by effector lymphocytes, but also critically influences the development and suppressive function of regulatory T-cells. Whereas, DcR3 restricts the function of the TL1A/DR3 complex: attenuating T-cell activation and downregulating the secretion of pro-inflammatory cytokines. Together with DR3 and DcR3, TL1A constitutes a cytokine system that actively interferes with the regulation of immune responses.

The Human TL1A-DR3 Binding Kit (TR-FRET) can detect the inhibitors of human TL1A binding to human DR3 in homogeneous system within 0.5-1 hours, it is highly sensitive, short detection time and easy to use.

PRINCIPLE OF THE ASSAY

This Human TL1A-DR3 inhibition kit (TR-FRET) is based on TR-FRET technology (Time-Resolved Fluorescence Resonance Energy Transfer). Use a mixture of biotinylated human TL1A and Europium-chelate labeled streptavidin as the donor and FA labeled human DR3 protein as the acceptor.

Your experiment will include 3 simple steps:

1) Mix the sample or Human Anti-TL1A Neutralizing Antibody in the kit with Human TL1A Protein Europium-chelate (Donor) and incubate at room temperature for 0.5 hours.

2) Add FA labeled Human DR3 Protein (Acceptor) and incubate at room temperature for at least 0.5 hours.

3) Use the TR-FRET module of a microplate reader to read the fluorescence signal at 665nm and 620nm. Calculate the Ratio based on the formula Ratio = $\frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$. The Ratio value is negatively correlated with the inhibitors in the sample.

— When the sample does not contain the inhibitors of human TL1A binding to human DR3, the donor and acceptor

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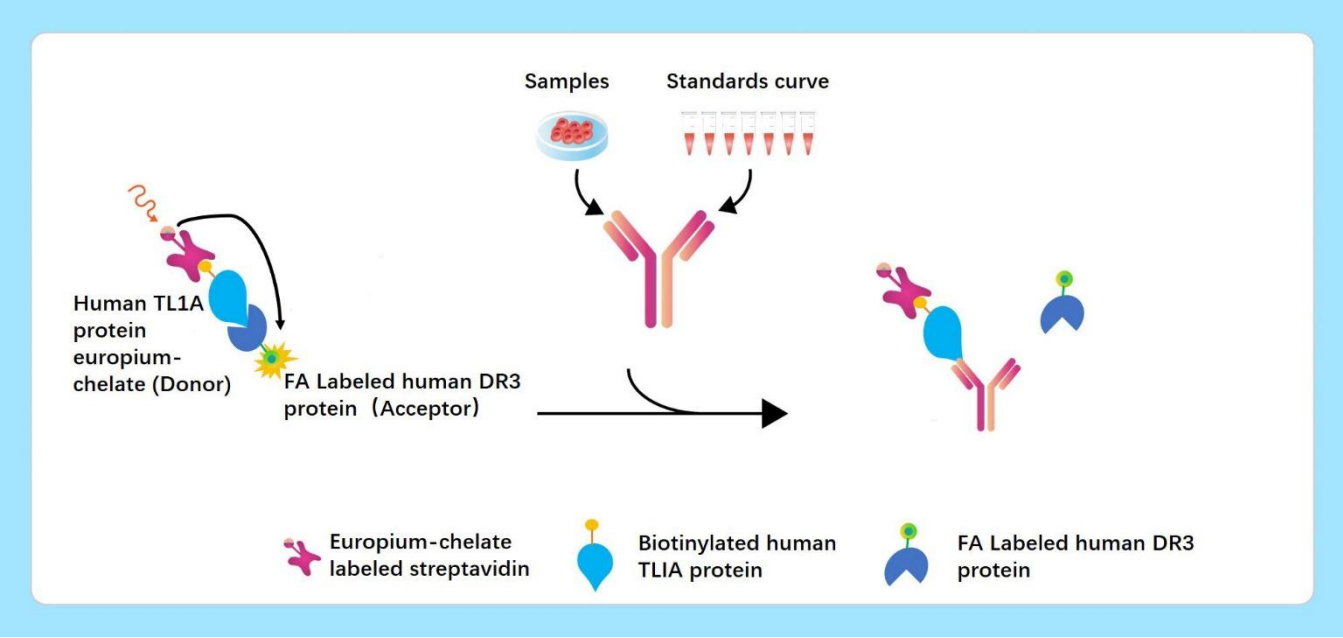
are in close proximity because of the binding of human TL1A and FA labeled human DR3. The 620 nm signal emitted by the donor under specific light source excitation is received by the acceptor, emitting a 665 nm signal.

— When the sample contains the inhibitors of human TL1A binding to human DR3, the inhibitors prevent the binding between the donor and acceptor and thereby prevent FRET from occurring.

FIG.1 PRINCIPLE OF THE ASSAY



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MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (100 tests)	Size (500 tests)	Format	Storage	
					Unopened	Opened
FRT02-C01	Human TL1A Protein Europium-chelate	100 tests	500 tests	Powder	2-8℃, avoid light	-70℃, avoid light
FRT02-C02	FA Labeled Human DR3 Protein	100 tests	500 tests	Powder	2-8℃, avoid light	-70℃, avoid light
FRT02-C03	Human Anti-TL1A Neutralizing Antibody	20 μg	100 μg	Powder	2-8℃	-70℃
FRT02-C04	Sample Dilution Buffer	10 mL	10 mL	Liquid	2-8℃	2-8℃
FRT02-C05	Detection Buffer	10 mL	10 mL	Liquid	2-8℃	2-8℃

MATERIALS REQUIRED BUT NOT PROVIDED

1. Single channel or multichannel pipettes with 10 μL, 200 μL and 1000 μL precision;
2. 10 μL, 200 μL and 1000 μL pipette tips;
3. Microporous plate shaker;
4. Microplate reader with TR-FRET module which can detect signals at 665nm/620nm;
5. Test Tubes;
6. Timer;
7. White plate (96 or 384-well low volume white plate): For example, HTRF 96-well, white plate, low volume (Revvity, Cat. No. 66PL96100); White Opaque 384-well Microplate (Perkinelmer, Cat.No. 6007299).
8. Deionized or distilled water for reconstitute;

STORAGE AND VALIDITY INSTRUCTIONS

1. Unopened kit should be stored at 2℃-8℃ upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

REAGENT PREPARATION

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

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2. Reconstitute the provided lyophilized materials to stock solutions with water as recommended in Table 2 and 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.

***Note**: Human TL1A Protein Europium-chelate and FA labeled Human DR3 Protein stock solution should be protected from light.*

TABLE 2. RECONSTITUTION METHODS FOR 100 TESTS AND 500 TESTS

Catalog	Components	Size (100 tests)		Size (500 tests)		Stock Solution Conc.
		Amount	Reconstitution Buffer and Vol.	Amount	Reconstitution Buffer and Vol.	
FRT02-C01	Human TL1A Protein Europium-chelate	100 tests	60 µL water	500 tests	300 µL water	About 15 µg/mL
FRT02-C02	FA Labeled Human DR3 Protein	100 tests	120 µL water	500 tests	600 µL water	About 400 µg/mL
FRT02-C03	Human Anti-TL1A Neutralizing Antibody	20µg	100 µL water	100µg	500 µL water	200 µg/mL

RECOMMENDED PROTOCOL

1. Add Samples

1.1 Make series dilution of the samples as appropriate.

1.2 If you intend to use the provided Human Anti-TL1A Neutralizing as a reference (Std.), you may dilute the antibody as recommend in FIG. 2. Dilute the sample to be tested appropriately using the Sample Dilution Buffer.

1.3 Add 10 µL of sample and standard solution to each well according to our recommendation (FIG. 3) or your own plate setup.

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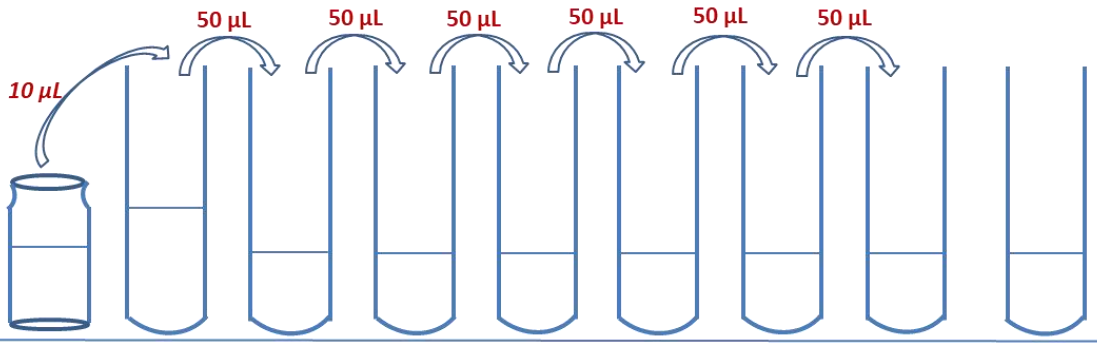
FIG.2 PREPARATION OF 1:2 SERIAL DILUTIONS OF THE HUMAN ANTI-TL1A NEUTRALIZING ANTIBODY

Tubes/ Solution Code	Anti-TL1A Neutralizing Antibody Stock Solution	Std 7	Std 6	Std 5	Std 4	Std 3	Std 2	Std 1	Std 0 (Blank)
Operating									
Solution Conc.	200 μg/mL	20 μg/mL	10 μg/mL	5 μg/mL	2.5 μg/mL	1.25 μg/mL	0.625 μg/mL	0.3125 μg/mL	0 μg/mL
Dilution Buffer Vol.		90 μL	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

2. Add Donor

Dilute **Human TL1A Protein Europium- chelate** stock solution 10-fold with **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.

3. Add Acceptor

Dilute **FA Labeled Human DR3 Protein** stock solution 5-fold with **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.

Refer to FIG. 3 and Table 3 for the design of microplate layout according to the experimental requirements, and add the corresponding reaction solution into the corresponding plate holes.

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TABLE 3. SAMPLES ADDING TO MICROPLATE

	1	2	3	4
A	10 μL Std7 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std7 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample1 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample1 5 μL Donor working solution 5 μL Acceptor working solution
B	10 μL Std6 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std6 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample2 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample2 5 μL Donor working solution 5 μL Acceptor working solution
C	10 μL Std5 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std5 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample3 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample3 5 μL Donor working solution 5 μL Acceptor working solution
D	10 μL Std4 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std4 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample Dilution Buffer 5 μL Donor working solution 5 μL Detection Buffer	10 μL Sample Dilution Buffer 5 μL Donor working solution 5 μL Detection Buffer
E	10 μL Std3 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std3 5 μL Donor working solution 5 μL Acceptor working solution	…	…
F	10 μL Std2 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std2 5 μL Donor working solution 5 μL Acceptor working solution	…	…
G	10 μL Std1 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std1 5 μL Donor working solution 5 μL Acceptor working solution	…	…
H	10 μL Sample Dilution Buffer 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample Dilution Buffer 5 μL Donor working solution 5 μL Acceptor working solution	…	…

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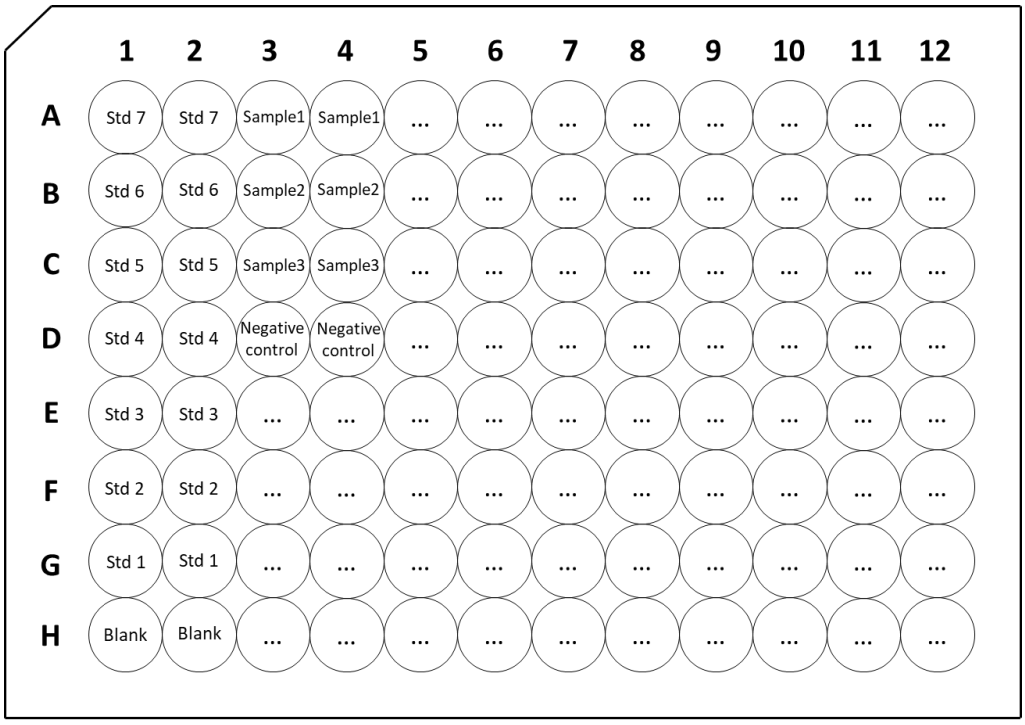
FIG.3 PLATE LAYOUT



4. Data Recording

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

5. Calculate Ratio

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

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
5. This kit should be stored at 2°C -8°C.
6. Please prepare the working solution of each component according to the needs of the experiment. All prepared working solution is for one-time use and cannot be stored.

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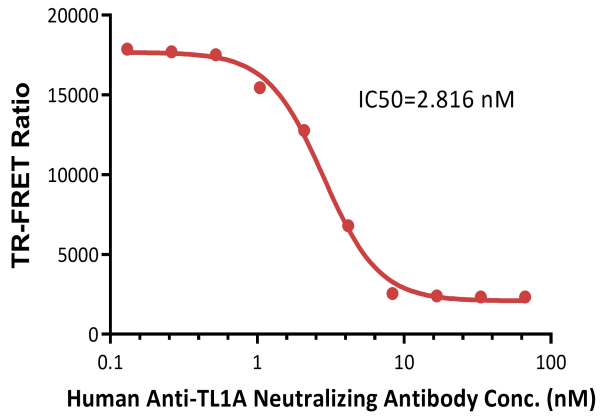
TYPICAL DATA

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.



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

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