CAT. & Size	A1070001S (1,000 tests)	VKEYBIO-02-2024
	A1070001L (10,000 tests)	For Research Use Only
Storage at	-60°C or below	Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET

Human TNFα Detection kit

Instruction Manual

1. Introduction

The KeyTec® TR-FRET Human TNFαassay kit is designed for quantitative measurement of Human TNFα protein in supernatant. It is based on sandwich immunoassay model and utilizes TR-FRET technology, known for its ease of use, homogeneity (no wash), low background, high sensitivity, robustness.

The detection principle is based on TR-FRET technology. A pair of mAb anti-TNFα antibodies, each labeled with KeyTec® TR-FRET Solar Eu[1] and KeyTec® TR-FRET LA[2], bind to the same antigen Human TNFα protein. This binding brings the donor molecule into proximity with the acceptor molecule. Excitation of the donor will result in the generation of the TR-FRET signal at 665 nm, proportional to the concentration of Human TNFα protein. (Figure 1)

*[1] KeyTec® TR-FRET Solar Eu: TR-FRET Donor Molecule

*[2] KeyTec® TR-FRET LA: TR-FRET Acceptor Molecule

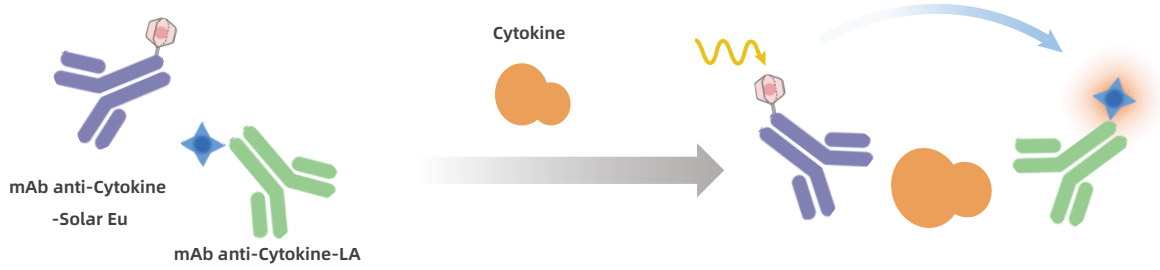

Figure 1. KeyTec® TR-FRET cytokine detection kit mode

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2. Components

Components	Storage	A1070001S (1,000 tests*3)	A1070001L (10,000 tests*3)
mAb anti-TNFα-Solar Eu (40X)	⩽ -60 °C	1 vial 50 µL/vial	1 vial 500 µL/vial
mAb anti-TNFα-LA (40X)	⩽ -60 °C	1 vial 50 µL/vial	1 vial 500 µL/vial
Human TNFα Standard	2-8 °C	2 vials Lyophilized	2 vials Lyophilized
Cytokine Diluent Buffer	2-8 °C	1 bottle 20 mL/bottle	1 bottle 50 mL/bottle
Cytokine Detection Buffer	2-8 °C	1 bottle 10 mL/bottle	1 bottle 50 mL/bottle

*3 The tests are sufficient in a 384-well microplate assay format, with 20 µL per well.

3. Storage Conditions

- Upon receipt, store the kit below -60 °C. Kit components remain stable under appropriate storage conditions as recommended.
- Once reconstituted, the standard stock solution may be stored below -60 °C. Aliquot the reagents as needed to avoid multiple freeze-thaw cycles.
- When first thaw, aliquot the components as needed to avoid multiple freeze-thaw cycles
- Volume of standard and antibody aliquots should not be under 10 µL.
- Up to 1 years from date of receipt, when stored and handled as recommended.

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4. Materials Required But Not Supplied

Materials	Recommended Brand	CAT.
ddH_2O	Multiple Choices	\
Microplates (KeyTec® 384-Well White Flat Low-Volume Microplates)	VKEY-BIO	M2000102N
KeyTec® Fluorescent High-Transparency Microplate Top Seals	VKEY-BIO	M1000102N
Pipettes	Multiple Choices	\
Microplate Reader With TR-FRET	Multiple Choices	\

5. Assay Procedure

5.1 Assay Format

Assay Format	Total Volume (20 µL*4)
Standard or Sample	16 µL
Pre-mixed TNFα Antibodies	4 µL

*4 The system accommodates 384-well microplates, and assay volumes can be adjusted proportionally to perform in 96- or 1536-well microplates.

5.2 Reagents Handling

1) Buffers

- Thaw the buffer solution at room temperature and equilibrate before use. The thawed buffer can be stored at 2-8 °C.
- Use the specified buffer to prepare reagents to avoid affecting assay results.

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2) Conjugates

- Thaw conjugates on ice, equilibrate to room temperature, and centrifuge before use. Avoid multiple freeze-thaw cycles.
- The stock solution for mAb anti-TNFα-Solar Eu is 40X; dilute 40 times with Cytokine Detection Buffer for a 1X working solution (2 µL per well). For example, mix 10 µL of the mAb anti-TNFα-Solar Eu stock solution with 390 µL of Cytokine Detection Buffer for a 400 µL 1X working solution.
- The stock solution for mAb anti-TNFα-LA is 40X; dilute 40 times with Cytokine Detection Buffer for a 1X working solution (2 µL per well). For example, mix 10 µL of the mAb anti-TNFα-LA stock solution with 390 µL of Cytokine Detection Buffer for a 400 µL 1X working solution.
- Mix the 1X working solutions of mAb anti-TNFα-Solar Eu and mAb anti-TNFα-LA in a 1:1 ratio for pre-mixed TNFα Antibodies[*5]. For example, mix 400 µL of the mAb anti- TNFα-LA 1X working solution with 400 µL of the mAb anti-TNFα-LA 1X working solution for 800 µL pre-mixed TNFα Antibodies 1X working solution.

[*5] It is recommended to use the pre-mixed Cytokine antibodies for testing to reduce operational steps and minimize deviations introduced by operations.

3) Samples

- Dilute the samples using Cytokine Diluent Buffer or the sample medium until their concentration falls within the quantifiable range of the standard curve (30 pg/mL – 5,000 pg/mL).

4) Standard

- **Reconstitute the TNFα standard with ddH_2O:** equilibrate to room temperature, and centrifuge the vial at 6,000 - 10,000 rpm for 10 - 20 seconds or 3,000 rpm for 2 - 3 minutes before use. Add ddH_2O as indicated on the label. Gently tap or invert the vial to ensure thorough dissolution if the lyophilized powder, avoiding vortex shaking. Allow the standard to sit at room temperature for more than 15 minutes to ensure complete dissolution. The redissolved standard samples can only undergo one freeze-thaw cycle.
- **Prepare the serial dilution standards solution:** Use the same buffer as the sample dilution buffer to prepare the standards following ***Table-1***. To minimize matrix effects and obtain more accurate sample concentrations, it is recommended to use the same buffer as the sample dilution buffer. For example, if the sample is diluted with cell culture supernatant cultured in DMEM medium + 10% FBS, then use the same medium (DMEM + 10% FBS) for diluting the standard. Determine the total amount of standard to be prepared based on assay requirements; the amounts in ***tale-1*** are for reference only.

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Table-1: Standard curve working solution preparation.

Standard	Working Concentration TNFα (pg/mL)	Serial Dilution
STD-7	5,000.0	40 µL Standard stock Solution + 160 µL Diluent Buffer
STD-6	2,272.7	100 µL STD-7 + 120 µL Diluent Buffer
STD-5	1,033.1	100 µL STD-6 + 120 µL Diluent Buffer
STD-4	469.6	100 µL STD-5 + 120 µL Diluent Buffer
STD-3	213.4	100 µL STD-4 + 120 µL Diluent Buffer
STD-2	97.0	100 µL STD-3 + 120 µL Diluent Buffer
STD-1	44.1	100 µL STD-2 + 120 µL Diluent Buffer
STD-0	0	120 µL Diluent Buffer

5.3 Procedure

- Add the reagents to the microplates in turn by following the steps shown in the following table.

	Negative Control*6	Standard Curve	Sample
Step 1	16 µL Diluent Buffer	16 µL Standard	16 µL sample
Step 2	4 µL pre-mixed TNFα Antibodies 1X working solution		
Step 3	Seal the microplate by "KeyTec® Fluorescent High-Transparency Microplate Top Seals" and incubate 1-3 hours at room temperature		
Step 4	(no need to remove the High-Transparency plate sealer) Read on the TR-FRET compatible reader		

*6 16 µL Diluent Buffer or the sample dilution buffer.

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5.4 Data Calculating

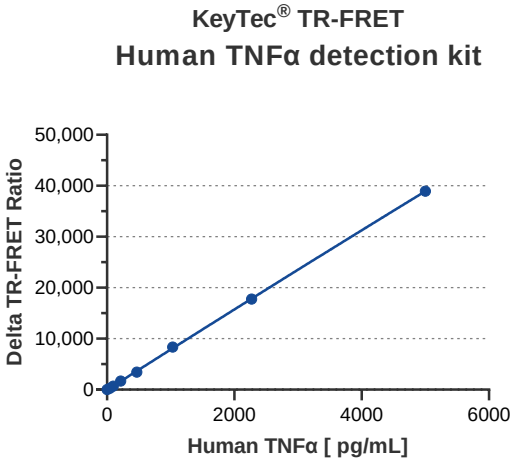
- Calculate the ratio of 665 nm/615 nm (TR-FRET Ratio) and the CV for each individual well.

$$\text{TR-FRET Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 615 nm}} \times 10{,}000$$

5.5 Performance

- Standard curve

Standard	TNFα (pg/mL)	TR-FRET Ratio	Delta Ratio	CV%
STD-7	5,000.0	40,879	39,532	0.4
STD-6	2,272.7	18,761	17,414	0.5
STD-5	1,033.1	9,674	8,327	2.9
STD-4	469.6	4,732	3,385	2.4
STD-3	213.4	3,121	1,774	3.4
STD-2	97.0	2,017	670	2.3
STD-1	44.1	1,634	287	0.5
STD-0	0	1,347	0	0.6

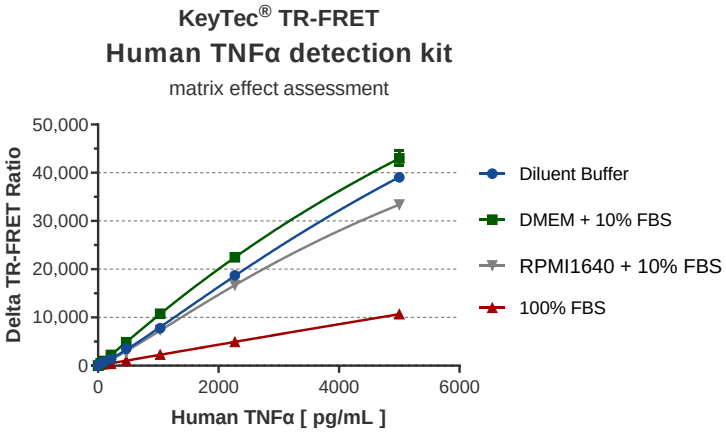


- performance

  Quantitative Range：30 pg/mL – 5,000 pg/mL

  Incubation Condition：Incubate at room temperature for 3 hours

- Effects of various matrices



Tip: The data provided above is for reference only. Actual results may vary depended on the performance of the microplate reader used.

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