

KeyTec® TR-FRET

Human IFN γ Detection kit



CAT.&Size: A1070012D(100 tests) A1070012S(1,000 tests)
A1070012L(10,000 tests)

Storage at: A1070012D(-60°C or Below) A1070012S/L(2-8°C)

VKEYBIO-01-2026
For Research Use Only

KeyTec® TR-FRET

Human IFN γ Detection Kit

Technical Manual

1. Introduction

KeyTec® TR-FRET Human IFN γ Detection Kit is designed for the quantitative determination of Human IFN γ in supernatants. This assay is based on a sandwich immunoassay method using KeyTec® TR-FRET technology, offering a simple, rapid, highly specific and sensitive, as well as reproducible detection process.

The principle, outlined in Figure 1: Two specific antibodies are conjugated to KeyTec® TR-FRET Solar Eu*¹ (Donor) and KeyTec® TR-FRET LA*² (Acceptor), respectively. When the two antibodies bind to different epitopes of Human IFN γ protein, the TR-FRET Donor and Acceptor are brought into close proximity. Excitation of the Donor with an external light source (laser or flash lamp) triggers Fluorescence Resonance Energy Transfer (FRET) to the Acceptor. The expression level of Human IFN γ protein is proportional to the signal intensity at a specific wavelength (665 nm).

*¹ KeyTec® TR-FRET Solar Eu: TR-FRET Donor

*² KeyTec® TR-FRET LA: TR-FRET Acceptor

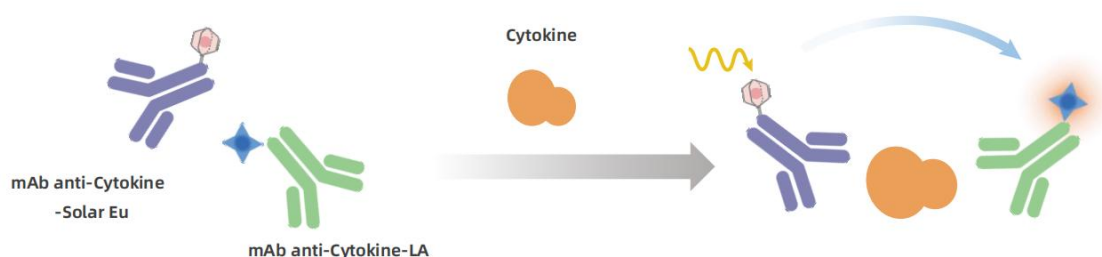


Figure1. The principle of KeyTec® TR-FRET Human IFN γ detection

2. Components

Components	Storage	A1070012D (100 tests ^{*3})	A1070012S (1,000 tests ^{*3})	A1070012L (10,000 tests ^{*3})
mAb anti-IFNγ-Solar Eu(400X)^{*4}	Lyophilized: 2-8°C Liquid: $\leq -60^{\circ}\text{C}$	1 vial (Liquid)	1 vial (Lyophilized)	1 vial (Lyophilized)
mAb anti-IFNγ-LA (400X)^{*4}	Lyophilized: 2-8°C Liquid: $\leq -60^{\circ}\text{C}$	1 vial (Liquid)	1 vial (Lyophilized)	1 vial (Lyophilized)
Human IFNγ Standard	Lyophilized: 2-8°C Liquid: $\leq -60^{\circ}\text{C}$	1 vial (Liquid) 80 ng/mL	2 vials (Lyophilized) A1070012N	2 vials (Lyophilized) A1070012N
Cytokine Diluent Buffer (Ready-to-Use)	2-8°C	2 vials 2 mL/vial A1010006D	1 bottle 20 mL/bottle A1010006S	1 bottle 50 mL/bottle A1010006L
Cytokine Detection Buffer (Ready-to-Use)	2-8°C	1 vial 2 mL/vial A1010007D	1 bottle 10 mL/bottle A1010007S	1 bottle 50 mL/bottle A1010007L

^{*3} Tests refer to the number of assay wells that can be performed in 96-well or 384-well plates with 20 μL in total reaction volume. The reagents of the kit are suggested to use as recommended.

^{*4} Stock concentration, final concentration is 1X in the 20 μL reaction system (refer to 5.1 Step 2).

3. Storage

- ◆ Store all reagents according to the recommended conditions. The products are stable for one year from the date of receipt.
- ◆ After reconstitution, the Human IFN γ Standard and detection antibodies must be stored at $\leq -60^{\circ}\text{C}$. Aliquot as needed to avoid multiple freeze-thaw cycles, the recommended aliquot volume is not less than 10 μL .

4. Required Components (Not Supplied)

Material	Brand	Catalog
Microplate(96-Well White Flat Low-Volume Microplates)	VKEY-BIO	M2000702N
Microplate(384-Well White Flat Microplates)	VKEY-BIO	M2000102N
Top sealing film	VKEY-BIO	M1000102N
Microplate Reader with TR-FRET module	Multiple Options	\

5. Reagent Preparation

5.1 Reaction system

Reaction Step	Volume(Total Volume ^{*5} :20 μ L)	Reagent Preparation
Step 1: Sample Preparation	16 μ L Sample or Standard (Working Solution)	Refer to Step 5.3.2 for sample preparation and Step 5.3.1 for standard preparation. Dilute samples using Cytokine Diluent Buffer or a matrix-matched buffer to ensure they fall within the quantifiable range of the standard curve (175 pg/mL - 10,000 pg/mL).
Step 2: Addition of Detection Reagent	4 μ L 5X Premixed Detection Antibody Pair	5X Premixed Detection Antibody Pair: Combine 1 volume of 400X Eu-labeled antibody, 1 volume of 400X LA-labeled antibody, and 78 volumes of Detection Buffer (Ready-to-Use). Mix gently.

^{*5} Recommended Format: Shallow-well 384-well microplate; For 96-well or 1536-well microplates, proportionally scale the reaction system.

5.2 Reagent preparation

- ◆ **Centrifuge lyophilized reagents (850 $\times$ g for 1-2 minutes) to collect powder at the bottom before reconstitution.** Powder Segregation is normal, especially during transport.

- ◆ Prior to use, thaw reagents on ice, equilibrate to room temperature, and mix liquid reagents thoroughly.
- ◆ Use buffers provided in the kit for dilution and preparation to ensure assay accuracy and stability.
- ◆ Prepare reagents according to kit technical manual.
- ◆ Prepare all reagents immediately before use, unless otherwise specified in the “Working Solution Preparation” section.
- ◆ Use KeyTec® TR-FRET detection reagents at recommended concentration. **Incorrect concentrations affect assay performance, stability, and reproducibility.** Mix all reagents gently; **do not vortex.**

5.3 Working Solution Preparation

5.3.1 Standard Preparation

- ◆ **Reconstitution of Standard:** Equilibrate the standard to room temperature. Centrifuge (850 ×g for 1-2 minutes) to collect powder at the bottom. Add ultrapure water to the volume indicated on the vial label. Do not vortex; invert gently to mix. Allow the standard to stand at room temperature for at least 15 minutes. **The reconstituted standard can be frozen and thawed only once.** The reconstituted stock concentration is 80 ng/mL.
- ◆ **Standard Curve Preparation:** Prepare serially diluted standards as shown in the table below. Use Cytokine Diluent Buffer or a solution with the same matrix as the test samples. For example, if test samples are cell supernatants cultured in DMEM medium containing 10% FBS, prepare standards in the same matrix (DMEM + 10% FBS).

Standard Curve	IFN γ Working Conc.(pg/mL)	Final IFN γ Conc. in 20 μ L Assay(pg/mL)	Preparation
STD-7	10,000.0	8,000.0	25 μ L Standard Stock Solution + 175 μ L Diluent Buffer
STD-6	4,000.0	3,200.0	80 μ L STD-7 + 120 μ L Diluent Buffer
STD-5	1,600.0	1,280.0	80 μ L STD-6 + 120 μ L Diluent Buffer
STD-4	640.0	512.0	80 μ L STD-5 + 120 μ L Diluent Buffer
STD-3	256.0	204.8	80 μ L STD-4 + 120 μ L Diluent Buffer
STD-2	102.4	81.9	80 μ L STD-3 + 120 μ L Diluent Buffer
STD-1	41.0	32.8	80 μ L STD-2 + 120 μ L Diluent Buffer
STD-0	0	0	120 μ L Diluent Buffer

5.3.2 Sample Preparation

- ◆ Dilute samples to within the range of 175 – 10,000 pg/mL. To minimize matrix interference and ensure optimal curve fitting, use the same matrix-matched buffer for both samples and standards.

5.3.3 Detection Antibodies Preparation

- ◆ **Reconstitution and Preparation of mAb anti-IFN γ -Solar Eu working solution(10X):** Centrifuge (850 $\times$ g for 1-2 minutes) to collect powder at the bottom before reconstitution. Add the appropriate volume of ultrapure water according to the kit size and mix gently to dissolve the lyophilized powder as the table below. The stock solution is 400X. Dilute 1 volume of stock solution with 39 volumes of Cytokine Detection Buffer to obtain the 10X working solution.
- ◆ **Reconstitution and Preparation of mAb anti-IFN γ -LA working solution(10X):** Centrifuge (850 $\times$ g for 1-2 minutes) to collect powder at the bottom before reconstitution. Add the appropriate volume of ultrapure water according to the kit size and mix gently to dissolve the lyophilized powder as the table below. The stock solution is 400X. Dilute 1 volume of stock solution with 39 volumes of Cytokine Detection Buffer to obtain the 10X working solution.

Detection Antibody Size	Reconstitution Buffer	Buffer Volume	Usage of Reconstituted Stock Solution
1,000 tests/vial	Ultrapure water	50 µL/vial	Dilute 40-fold with Cytokine Detection Buffer before use
10,000 tests/vial	Ultrapure water	500 µL/vial	Dilute 40-fold with Cytokine Detection Buffer before use

- ◆ **Preparation of Premixed Detection Antibody Pair(5X):** Mix the prepared mAb anti-IFN γ -Solar Eu working solution and mAb anti-IFN γ -LA working solution at a 1:1 ratio to obtain the 5X premixed detection antibody pair^{*6}. Add 4 µL per well.

^{*6} This pre-mix mode reduces operational steps and deviations.

6. Procedure

- ◆ Follow the steps in the table below.

	Negative Control ^{*7}	Standard Curve	Test samples
Step 1	16 µL Diluent Buffer	16 µL serially diluted standards	16 µL prepared test samples
Step 2	4 µL Premixed Detection Antibody Pair		
Step 3	Seal the microplate to prevent evaporation. Incubate 2 hours to overnight at room temperature (25°C).		
Step 4	Read on a TR-FRET compatible reader (no need to remove the seals).		

^{*7} 16 µL Diluent Buffer or a solution with the same matrix as the samples.

7. Data Analysis

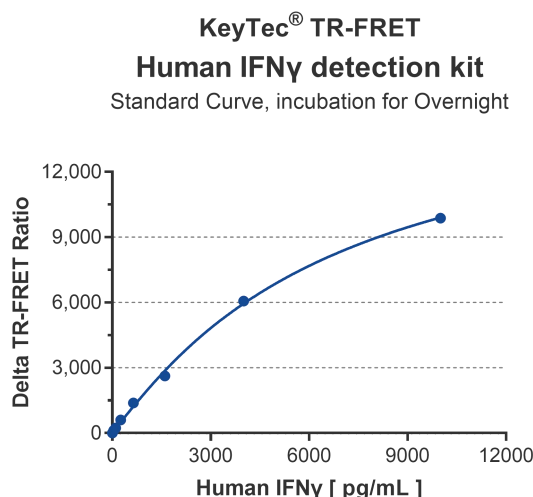
- ◆ Calculate the 665 nm/620 nm Ratio(TR-FRET Ratio) and the percentage coefficient of variation (CV %) for each well.

$$\text{TR-FRET Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10,000$$

8. Results

◆ Standard Curve

Standard	IFN γ (pg/mL)	TR-FRET Ratio	Delta Ratio	CV%
STD-7	10,000.0	10,427	9,877	4.0
STD-6	4,000.0	6,613	6,063	4.3
STD-5	1,600.0	3,170	2,620	4.4
STD-4	640.0	1,937	1,387	2.6
STD-3	256.0	1,156	606	3.7
STD-2	102.4	780	230	2.1
STD-1	41.0	677	127	5.6
STD-0	0	550	0	3.0



◆ Assay Performance

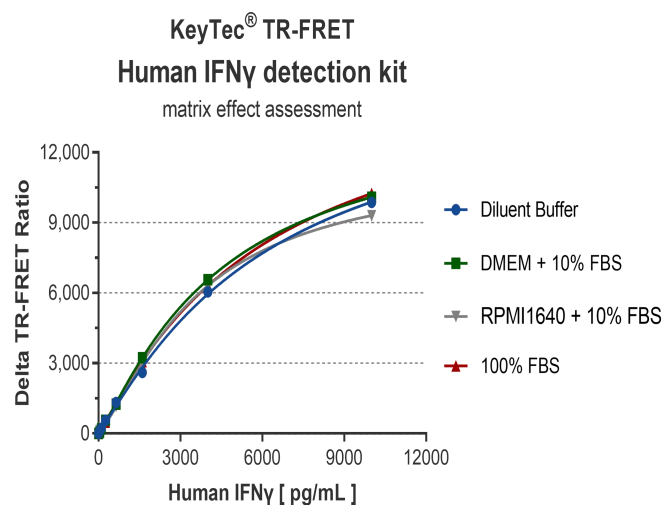
Quantification Range: 175 pg/mL – 10,000 pg/mL

Incubation time: 2 hours to overnight at room temperature (25°C).

Performance Parameter	Diluent buffer	RPMI1640 +10% FBS	DMEM +10% FBS	100% FBS
Assay range (pg/mL)		175.0 pg/mL – 10,000 pg/mL		
Limit of detection (LoD ^{*8}) [Std 0 Mean + 2×SD]	31.5 pg/mL	38.0 pg/mL	49.0 pg/mL	143.5 pg/mL
Limit of quantification (LoQ ^{*8}) [Std 0 Mean + 10×SD]	174.5 pg/mL	201.4 pg/mL	211.5 pg/mL	528.1 pg/mL
Incubation time	Overnight at room temperature			

^{*8} The data are calculated from Overnight readings on a specific microplate reader with optimized parameters. Results may vary depending on microplate reader performance.

◆ Assessment of Matrix Effect



Note: Exemplary data are shown. Results are instrument-dependent.

9. Instrument Model and Setting

Vendor	TECAN
Instrument model	Infinite® 200 PRO [Ref. 30050303]
Mode	Fluorescence Top Reading
Excitation filter	320 (25) nm [Ref. 30094454]
Emission filter 1	665 (8.5) nm [Ref. 30094518]
Emission filter 2	620 (10) nm [Ref. 30094505]
Mirror	Dichroic 510
Lag time	150 μ s
Integration Time	500 μ s
Number of reads	5 or user-defined
Gain	150 or optimal
Z -focus (mm)	Can be calculated on the well giving the highest signal