CAT.&Size: A1070004S (1,000 tests)
A1070004L (10,000 tests)
Storage at: 2-8 ℃

VKEYBIO-01-2025
For Research Use Only
Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET
Human IL8 Detection Kit
Technical Manual

1. Introduction

KeyTec® TR-FRET Human IL8 Detection Kit is designed for the quantitative determination of Human IL8 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 IL8 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 IL8 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

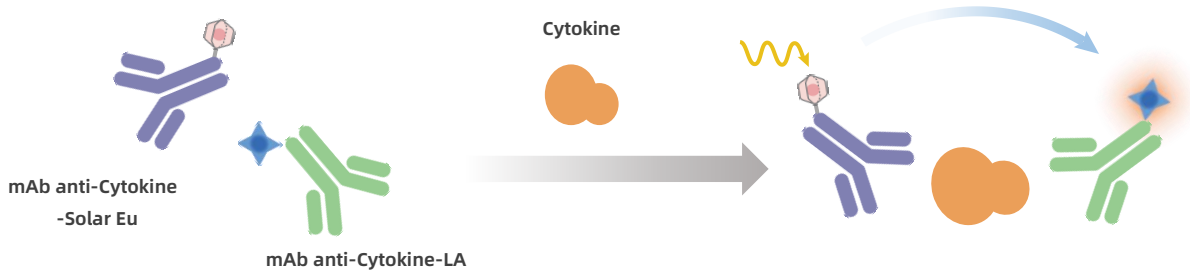


Figure1. The principle of KeyTec® TR-FRET Human IL8 detection

Shanghai VKEY Biotechnologies Co., Ltd.
F3, Building 2, Lane 898, Halei Road, Pudong New Area, Shanghai, China
Website: www.vkeybio.com

For technical support, please contact VKEY-BIO
E-mail: technical-support@vkeybio.com
Tel.: 021-5835-6939

2. Components

Components	Storage	A1070004S (1,000 tests*3)	A1070004L (10,000 tests*3)
mAb anti-IL8 - Solar Eu (Lyophilized)	2-8 ℃	1 vial 1,000 tests/vial	1 vial 10,000 tests/vial
mAb anti-IL8 - LA (Lyophilized)	2-8 ℃	1 vial 1,000 tests/vial	1 vial 10,000 tests/vial
Human IL8 Standard (Lyophilized)	2-8 ℃	2 vials A1070004N	2 vials A1070004N
Cytokine Diluent Buffer	2-8 ℃	1 bottle 20 mL/bottle	1 bottle 50 mL/bottle
Cytokine Detection Buffer	2-8 ℃	1 bottle 10 mL/bottle	1 bottle 50 mL/bottle

*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.

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 IL8 Standard and detection antibodies must be stored at ≤-60 ℃. Aliquot as needed to avoid multiple freeze-thaw cycles, the recommended aliquot volume is not less than 10 µL.

Shanghai VKEY Biotechnologies Co., Ltd.
F3, Building 2, Lane 898, Halei Road, Pudong New Area, Shanghai, China
Website：www.vkeybio.com

For technical support, please contact VKEY-BIO
E-mail：technical-support@vkeybio.com
Tel.：021-5835-6939

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

Components	Volume(Total Volume*4:20 μL)
Test samples	16 μL
Premixed Detection Antibody Pair	4 μL

*4 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 ×g for 1-2 minutes) to collect powder at the bottom before reconstitution.** Powder Segregation is normal, especially during transport.
- Thaw reagents on ice; equilibrate to room temperature before use.
- 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.**

Shanghai VKEY Biotechnologies Co., Ltd.
F3, Building 2, Lane 898, Halei Road, Pudong New Area, Shanghai, China
Website: www.vkeybio.com

For technical support, please contact VKEY-BIO
E-mail: technical-support@vkeybio.com
Tel.: 021-5835-6939

6. Working Solution Preparation

6.1 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. Keep the standard at room temperature for more than 15 minutes to ensure complete dissolution. **The reconstituted standard can be frozen and thawed only once.** The reconstituted stock concentration is 100 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	IL8 Concentration (pg/mL)	Preparation
STD-7	10,000.0	20 µL Standard Stock Solution + 180 µL Diluent Buffer
STD-6	4,000.0	80 µL STD-7 + 120 µL Diluent Buffer
STD-5	1,600.0	80 µL STD-6 + 120 µL Diluent Buffer
STD-4	640.0	80 µL STD-5 + 120 µL Diluent Buffer
STD-3	256.0	80 µL STD-4 + 120 µL Diluent Buffer
STD-2	102.4	80 µL STD-3 + 120 µL Diluent Buffer
STD-1	41.0	80 µL STD-2 + 120 µL Diluent Buffer
STD-0	0.0	120 µL Diluent Buffer

6.2 Sample Preparation

- Dilute samples with Cytokine Diluent Buffer or a solution with the same matrix as the samples.

Shanghai VKEY Biotechnologies Co., Ltd.
F3, Building 2, Lane 898, Halei Road, Pudong New Area, Shanghai, China
Website: www.vkeybio.com

For technical support, please contact VKEY-BIO
E-mail: technical-support@vkeybio.com
Tel.: 021-5835-6939

6.3 Detection Antibodies Preparation

- **Preparation of mAb anti-IL8 - Solar Eu working solution(1X):** Centrifuge (850 ×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 40X. Dilute 1 volume of stock solution with 39 volume of Cytokine Detection Buffer.
- **Preparation of mAb anti-IL8 - LA working solution(1X)**: Centrifuge (850 ×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 40X. Dilute 1 volume of stock solution with 39 volume of Cytokine Detection Buffer.

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**: Mix the prepared mAb anti-IL8 - Solar Eu working solution and mAb anti-IL8 - LA working solution at a 1:1 ratio to obtain the premixed detection antibody pair[*5].

[*5] This pre-mix mode reduces operational steps and deviations.

Shanghai VKEY Biotechnologies Co., Ltd.
F3, Building 2, Lane 898, Halei Road, Pudong New Area, Shanghai, China
Website: www.vkeybio.com

For technical support, please contact VKEY-BIO
E-mail: technical-support@vkeybio.com
Tel.: 021-5835-6939

7. Procedure

- Follow the steps in the table below.

	Negative Control[*6]	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 1 hour to overnight at room temperature (25 °C).		
Step 4	Read on a TR-FRET compatible reader (no need to remove the seals).		

[*6] 16 µL Diluent Buffer or a solution with the same matrix as the samples.

8. Data Analysis

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

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

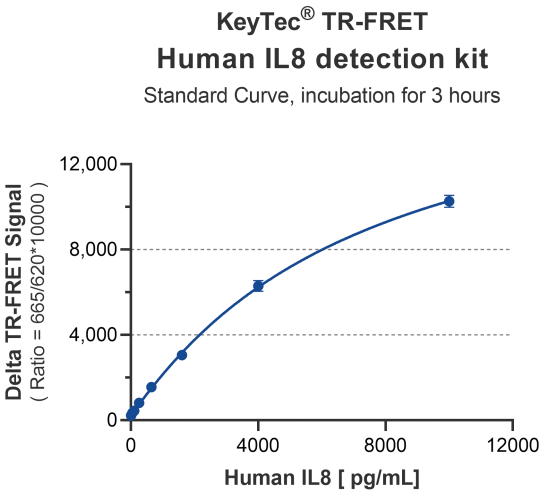
Shanghai VKEY Biotechnologies Co., Ltd.
F3, Building 2, Lane 898, Halei Road, Pudong New Area, Shanghai, China
Website: www.vkeybio.com

For technical support, please contact VKEY-BIO
E-mail: technical-support@vkeybio.com
Tel.: 021-5835-6939

9. Results

- Standard Curve

Standard	IL8 (pg/mL)	TR-FRET Ratio	Delta Ratio	CV%
STD-7	10,000	10,267	10,040	3.8
STD-6	4000.0	6,292	6,065	5.6
STD-5	1,600.0	3,050	2,824	10.2
STD-4	640.0	1,558	1,332	4.6
STD-3	256.0	816	589	0.2
STD-2	102.4	444	217	1.8
STD-1	41.0	349	122	1.8
STD-0	0	227	0	5.6



- Analytical Assay Performance

Quantification Range: 89 pg/mL – 10,000 pg/mL

Incubation time: 1 hour to overnight at room temperature (25 ℃).

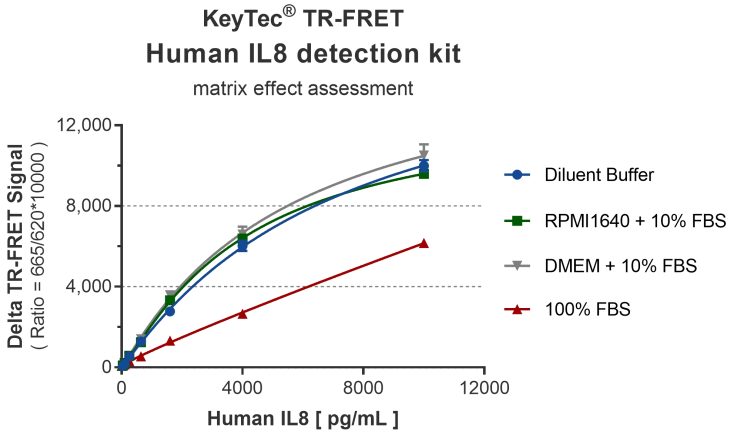
Performance Parameter	Diluent buffer	RPMI1640 +10% FBS	DMEM +10% FBS	100% FBS
Assay range (pg/mL)	89 pg/mL - 10,000 pg/mL			
Limit of detection (LoD*[7]) [Std 0 Mean + 2×SD]	15.96 pg/mL	25.46 pg/mL	34.46 pg/mL	78.90 pg/mL
Limit of quantification (LoQ*[7]) [Std 0 Mean + 10×SD]	88.68 pg/mL	115.4 pg/mL	149.4 pg/mL	570.3 pg/mL
Incubation time	3 hours at room temperature			

*7 The data are calculated from 3-hour readings on a specific microplate with optimized parameters. Results may vary depending on microplate reader performance.

Shanghai VKEY Biotechnologies Co., Ltd.
F3, Building 2, Lane 898, Halei Road, Pudong New Area, Shanghai, China
Website: www.vkeybio.com

For technical support, please contact VKEY-BIO
E-mail: technical-support@vkeybio.com
Tel.: 021-5835-6939

- Assessment of Matrix Effect



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

10. 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 μs
Integration Time	500 μs
Number of reads	5 or user-defined
Gain	150 or optimal
Z -focus (mm)	Can be calculated on the well giving the highest signal

Shanghai VKEY Biotechnologies Co., Ltd.
F3, Building 2, Lane 898, Halei Road, Pudong New Area, Shanghai, China
Website：www.vkeybio.com

For technical support, please contact VKEY-BIO
E-mail：technical-support@vkeybio.com
Tel.：021-5835-6939