CAT. & Size	A1040002S (1,000 tests)	VKEYBIO-01-2024
	A1040002L (10,000 tests)	For Research Use Only
Storage at	-60°C or below	Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET
Human Fc detection kit
Instruction Manual

1. Introduction

The KeyTec® TR-FRET Human Fc detection kit is designed for quantitative measurement of human IgG or human Fc-tagged protein in supernatant. It is based on competitive immunoassay model and utilizes TR-FRET technology, known for its ease of use, homogeneity (no wash), low background, wide range, robustness.

The screening principle is based on TR-FRET technology. The mAb anti-hFc antibody is labeled with KeyTec® TR-FRET Solar Eu*1 and Human IgG is labeled with KeyTec® TR-FRET LA*2, when mAb anti-hFc-Solar Eu binds to Human IgG-LA, the donor molecule is brought into proximity with the acceptor molecule. Excitation of the donor will result in the generation of the TR-FRET signal at 665 nm. Native human IgG or human Fc-tagged protein competes with the Human IgG-LA for binding to anti-hFc antibodies. The specific signal is inversely proportional to the concentration of human IgG or human Fc-tagged protein in the standard or sample. (Figure 1)

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

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

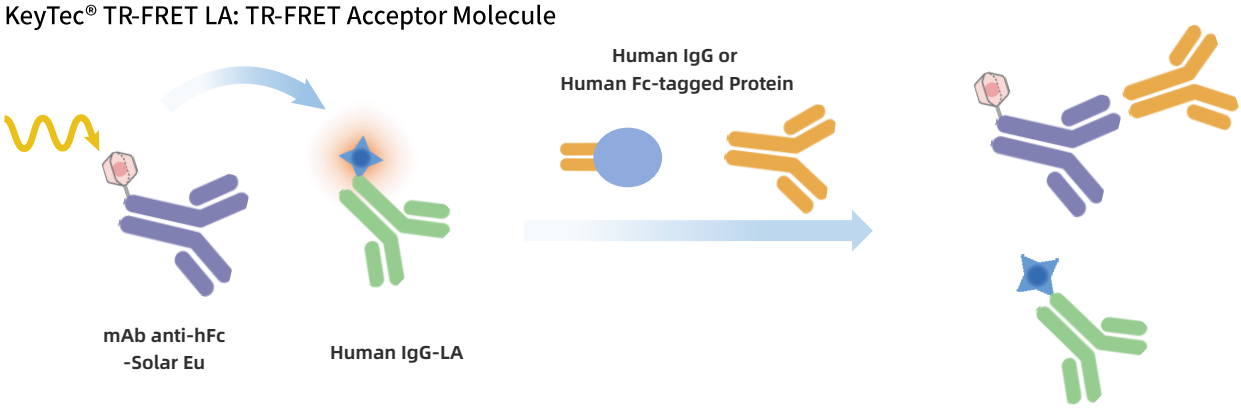

Figure 1. KeyTec® TR-FRET Human Fc detection kit mode

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2. Components

Components	Storage	A1040002S (1,000 tests*3)	A1040002L (10,000 tests*3)
mAb anti-hFc-Solar Eu (50X)	⩽ -60 °C	1 vial 100 µL/vial	1 vial 1 mL/vial
Human IgG-LA (50X)	⩽ -60 °C	1 vial 100 µL/vial	1 vial 1,000 µL/vial
Human IgG Standard (300 µg/mL)	⩽ -60 °C	1 vial 150 µL/vial	2 vials 150 µL/vial
Biotherapeutics Diluent Buffer	2-8 °C	1 bottle 30 mL/bottle	1 bottle 200 mL/bottle
Biotherapeutics Detection Buffer	2-8 °C	1 bottle 10 mL/bottle	1 bottle 100 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.
- 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.

4. Materials Required But Not Supplied

Materials	Recommended Brand	CAT.
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	\

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5. Assay Procedure

5.1 Assay Format

Assay Format	Total Volume (20 µL*4)
Human IgG Sample or Standard	10 µL
mAb anti-hFc-Solar Eu	5 µL
Human IgG-LA	5 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.

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-hFc-Solar Eu is 50X; dilute 50 times with Biotherapeutics Detection Buffer for a 1X working solution (5 µL per well). For example, mix 10 µL of the mAb anti-hFc-Solar Eu stock solution with 490 µL of Biotherapeutics Detection Buffer for a 500 µL 1X working solution.
- The stock solution for Human IgG-LA is 50X; dilute 50 times with Biotherapeutics Detection Buffer for a 1X working solution (5 µL per well). For example, mix 10 µL of the Human IgG-LA stock solution with 490 µL of Biotherapeutics Detection Buffer for a 500 µL 1X working solution.

3) Samples

- Dilute the samples using Biotherapeutics Diluent Buffer or the sample medium until their concentration falls within the quantifiable range of the standard curve (50 ng/mL –50 µg/mL).

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4) Standard

- **Prepare the serial dilution standards solution:** Use Biotherapeutics Diluent Buffer or the sample medium 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 ***table-1*** are for reference only.

Table-1: Standard curve working solution preparation.

Standard	Working Concentration IgG (ng/mL)	Serial Dilution
STD-8	60,000	24 µL Standard stock Solution + 96 µL Diluent Buffer
STD-7	20,000	40 µL STD-8 + 80 µL Diluent Buffer
STD-6	6,666.7	40 µL STD-7 + 80 µL Diluent Buffer
STD-5	2,222.2	40 µL STD-6 + 80 µL Diluent Buffer
STD-4	740.7	40 µL STD-5 + 80 µL Diluent Buffer
STD-3	246.9	40 µL STD-4 + 80 µL Diluent Buffer
STD-2	82.3	40 µL STD-3 + 80 µL Diluent Buffer
STD-1	27.4	40 µL STD-2 + 80 µL Diluent Buffer
STD-0 (PC)	0	80 µL Diluent Buffer
NC	0	See below for details*4

*4 Negative control (NC) : 10 µL Diluent Buffer or sample dilution buffer + 5 µL Detection Buffer + 5 µL 1X mAb anti-hFc-Solar Eu.

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5.3 Procedure

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

	Negative Control	Standard Curve	Sample
Step 1	10 µL Diluent Buffer*5	10 µL Standard	10 µL sample
Step 2	5 µL Detection Buffer	5 µL Human IgG-LA (1X)	5 µL Human IgG-LA (1X)
Step 3	5 µL mAb anti-hFc - Solar Eu (1X)		
Step 4	Seal the microplate by "KeyTec® Fluorescent High-Transparency Microplate Top Seals" and incubate 2 hours at room temperature		
Step 5	(no need to remove the High-Transparency plate sealer) Read on the TR-FRET compatible reader		

*5 10 µL Diluent Buffer or sample dilution buffer

5.4 Data Calculating

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

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

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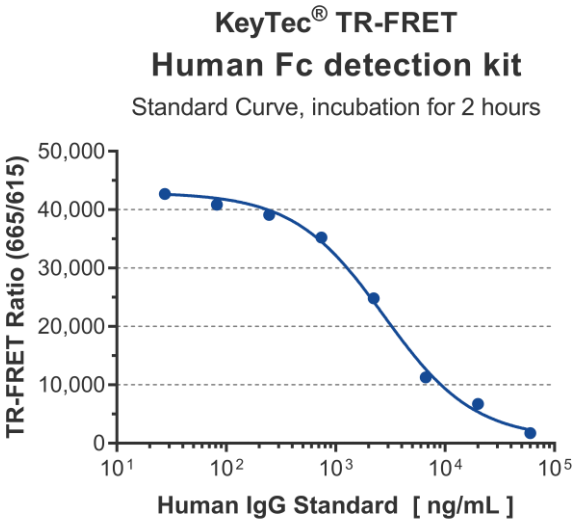
5.5 Performance

- Standard Curve

Standard	IgG (ng/mL)	TR-FRET Ratio	CV%
NC	\	839	5.0
STD-8	60,000	2,585	3.5
STD-7	20,000	7,551	1.6
STD-6	6,666.7	12,130	0.4
STD-5	2,222.2	25,635	1.6
STD-4	740.7	36,047	0.7
STD-3	246.9	39,939	3.0
STD-2	82.3	41,678	1.2
STD-1	27.4	43,547	1.6
STD-0	0	44,924	1.6

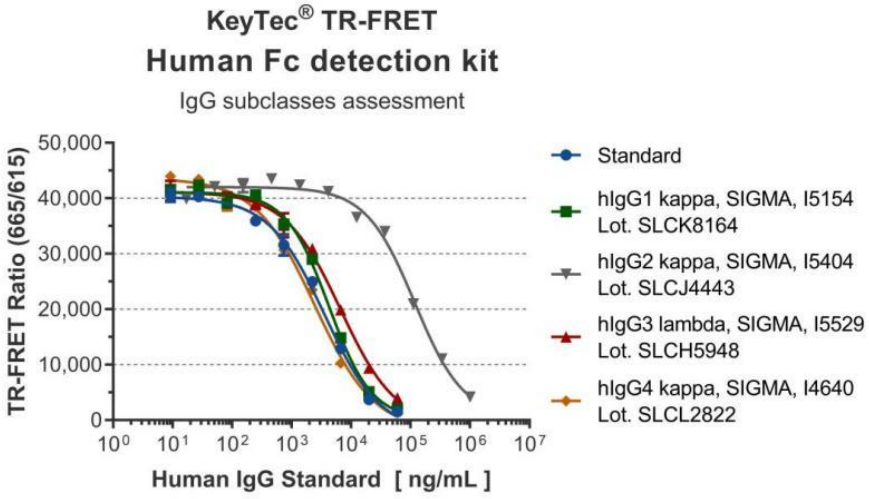


- Performance

Quantitative Range：50 ng/mL – 50, 000 ng/mL

Incubation Condition：Incubate at room temperature for 2 hours

- Results of various IgG subclasses



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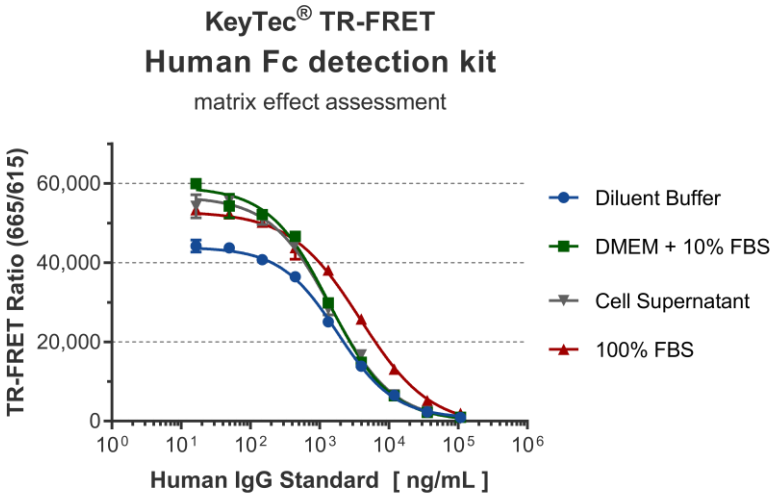
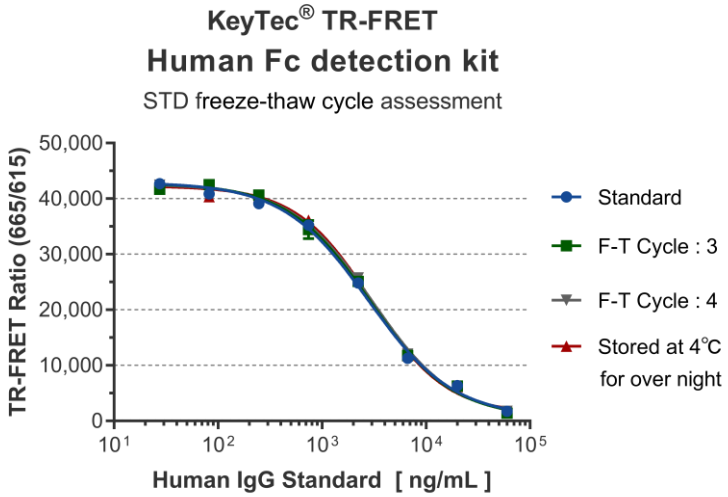
- Effects of various matrices



- Results of freeze-thaw and storage conditions of standard



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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