CAT. & Size	A1040001S (1,000 tests)	VKEYBIO-01-2024
	A1040001L (10,000 tests)	For Research Use Only
Storage at	-60°C or below	Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET
Human IgG detection kit
Instruction Manual

1. Introduction

The KeyTec® TR-FRET Human IgG detection kit is designed for quantitative measurement of human IgG 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 screening principle is based on TR-FRET technology. The mAb anti-hFc antibody is labeled with KeyTec® TR-FRET Solar Eu*1 and pAb anti-hFab antibody is labeled with KeyTec® TR-FRET LA*2, when mAb anti-hFc-Solar Eu and pAb anti-hFab-LA bind to Human IgG, 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, proportional to the concentration of Human IgG. (Figure 1)

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

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

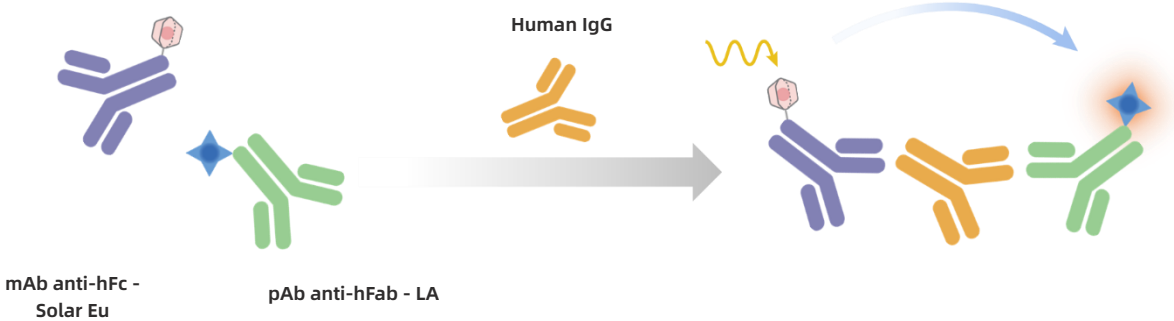

Figure 1. KeyTec® TR-FRET Human Fc detection kit mode

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2. Components

Components	Storage	A1040001S (1,000 tests*3)	A1040001L (10,000 tests*3)
mAb anti-hFc-Solar Eu (50X)	⩽ -60 °C	1 vial 100 µL/vial	1 vial 1,000 µL/vial
pAb anti-hFab-LA (50X)	⩽ -60 °C	1 vial 100 µL/vial	1 vial 1,000 µL/vial
Human IgG Standard (5 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
pAb anti-hFab-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 PAb anti-hFab-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 PAb anti-hFab-LA stock solution with 490 µL of Biotherapeutics Detection Buffer for a 500 µL 1X working solution.
- Mix the 1X working solutions of mAb anti-hFc-Solar Eu and pAb anti-hFab-LA in a 1:1 ratio.

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 (2 ng/mL-900 ng/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	1,000	25 µL Standard+ 100 µL Diluent Buffer
STD-7	400	50 µL STD-8 + 75 µL Diluent Buffer
STD-6	160	50 µL STD-7 + 75 µL Diluent Buffer
STD-5	64.0	50 µL STD-6 + 75 µL Diluent Buffer
STD-4	25.6	50 µL STD-5 + 75 µL Diluent Buffer
STD-3	10.2	50 µL STD-4 + 75 µL Diluent Buffer
STD-2	4.10	50 µL STD-3 + 75 µL Diluent Buffer
STD-1	1.64	50 µL STD-2 + 75 µL Diluent Buffer
STD-0 (NC)	0	75 µL Diluent Buffer

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5.3 Procedure

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

	Buffer Control*4	Solar Eu Control*5	Standard Curve (include NC)	Sample
Step 1	10 µL Diluent Buffer	10 µL Diluent Buffer	10 µL Standard	10 µL sample
Step 2	10 µL Detection Buffer	5 µL Detection Buffer	10 µL pre-mixed Antibodies 1X working solution*6	
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			

*4 Buffer Control: Used to assess the fluorescence background. It's recommended to use this setting for the initial assay setup.

*5 Solar Eu Control: Used to assess the signal of TR-FRET donor at 615 nm (or 620 nm). It's recommended to use this setting for the initial assay setup.

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

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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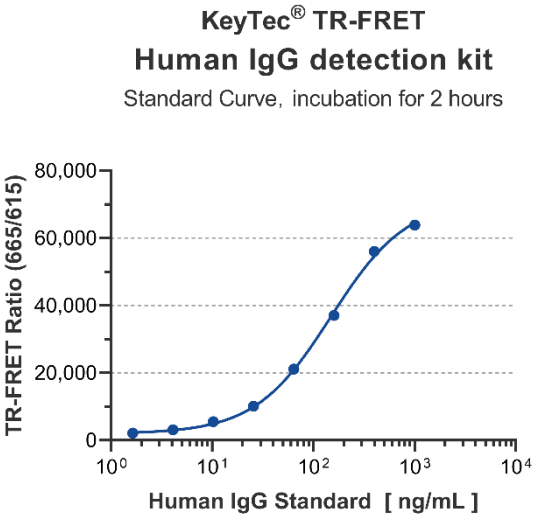
5.5 Performance

- Standard Curve

Standard	IgG (ng/mL)	TR-FRET Ratio	CV%
STD-8	1,000	63,891	2.5
STD-7	400	56,080	1.6
STD-6	160	37,072	1.4
STD-5	64.0	21,141	0.5
STD-4	25.6	10,156	0.1
STD-3	10.2	5,538	3.4
STD-2	4.10	3,140	1.3
STD-1	1.64	2,156	2.1
STD-0	0	1,461	0.3
STD-8	1,000	63,891	2.5

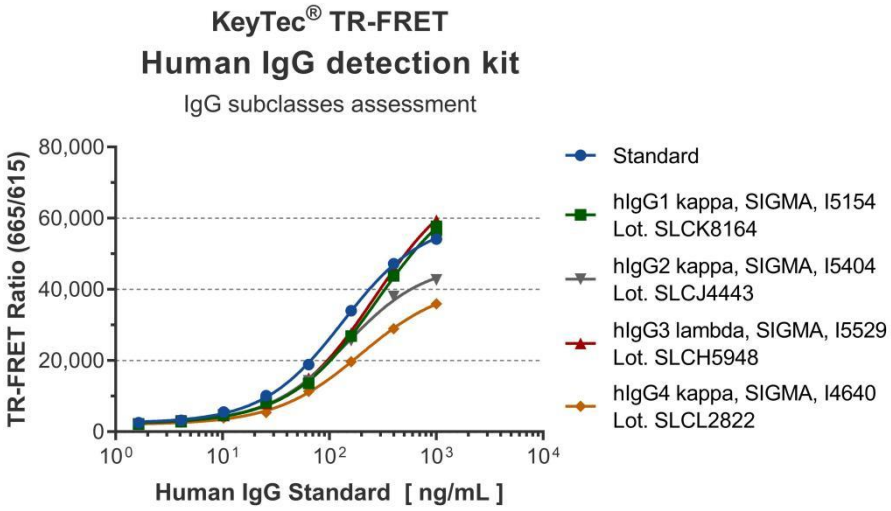


- Performance

Quantitative Range: 2 ng/mL – 900 ng/mL

Incubation Condition: Incubate at room temperature for 2 hours

- Results of various IgG subclasses



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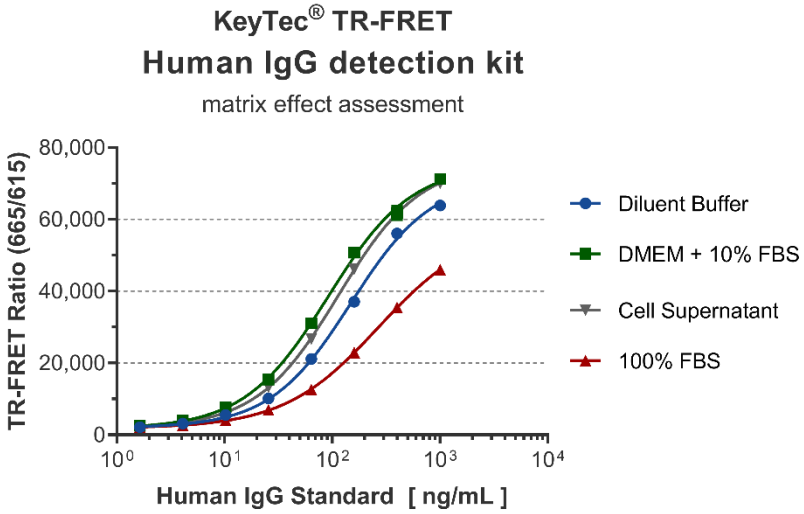
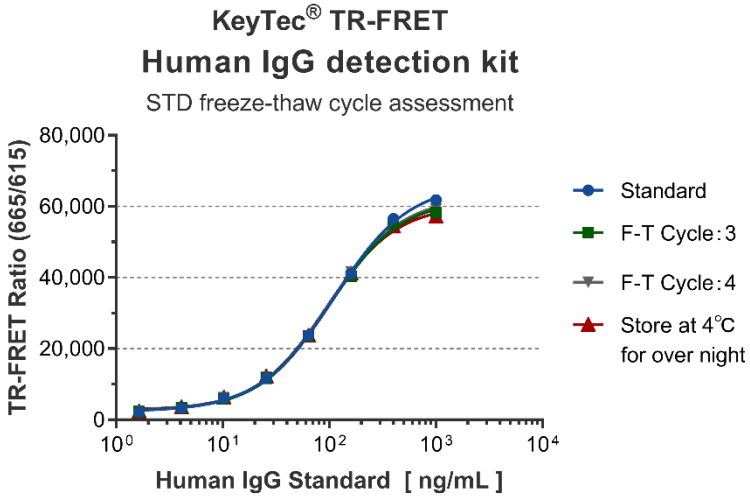
- Effects of various matrices



- 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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