CAT.&Size: A1040012S (500 tests)
A1040012L (5,000 tests)
Storage at: -60℃ or Below

VKEYBIO-02-2025
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Not For Diagnostic or Therapeutic Use

KeyTec® TR-FRET
Human Human FcRn Binding Kit
Technical Manual

1. Introduction

KeyTec® TR-FRET Human FcRn Binding Kit is designed to screen IgGs and FcRn inhibitors that bind to Human FcRn, and provides the ideal solution to assess the half-life of different IgGs. This assay is based on a competitive immunoassay method using KeyTec® TR-FRET technology, offering a simple, rapid, highly specific and sensitive, as well as reproducible detection process. The principle is outlined in Figure 1.

The ready-to-use pre-mix of KeyTec® TR-FRET Solar Eu[1]- Streptavidin and Biotinylated Human FcRn protein can bind to KeyTec® TR-FRET LA[2]- Human IgG, bringing Solar Eu (referred as donor) and LA (referred as acceptor) close to each other. Under excitation by an external light source, resonance energy transfer occurs between the donor and the acceptor. The binding affinity between FcRn and Human IgG can be determined by detecting the signal intensity at a specific wavelength (665 nm). Free Human IgGs or Human Fc-tagged proteins in the sample can compete with Human IgG-LA for the binding sites on FcRn. The TR-FRET signal intensity is inversely proportional to the concentration of free Human IgGs or Human Fc-tagged proteins in the sample.

*[1] KeyTec® TR-FRET LA: TR-FRET Acceptor

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

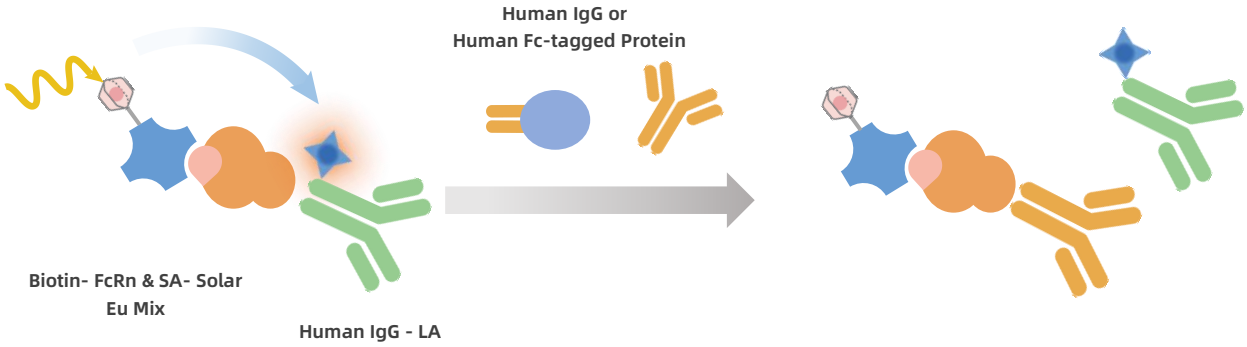


Figure 1. The Principle of KeyTec® TR-FRET Human FcRn Binding Assay

2. Components

Components	Storage	A1040012S (500 tests[*3])	A1040012L (5,000 tests[*3])
Biotin-FcRn & SA-Solar Eu Mix (100X)	≤ -60 ℃	1 vial 25 μL/vial	1 vial 250 μL/vial
Human IgG - LA（100X）	≤ -60 ℃	1 vial 25 μL/vial	1 vial 250 μL/vial
Human IgG（4 mg/ml）	≤ -60 ℃	1 vial 120 μL/vial	2 vial 120 μL/vial
FcRn Assay Diluent Buffer	2-8 ℃	1 bottle 50 mL/bottle A1010045S	1 bottle 200 mL/bottle A1010045L
FcRn Detection Buffer	2-8 ℃	1 bottle 30 mL/bottle A1010044S	1 bottle 120 mL/bottle A1010044L

[*3] The number of tests refers to performed in a low-volume 96-well or 384-well assay plate with a total reaction volume of 20 μL and reagents used at the concentrations as recommended.

3. Storage

- Store all reagents according to the recommended conditions. The products are stable for one year from the date of receipt.
- Store the reagents at -60 ℃ or below as indicated by the label. After thawing, aliquot the stock into single-use volumes to avoid repeated freeze-thaw cycles. The recommended aliquot volume is not less than 10 μL.

4. Required but Not Provided Materials and Equipment

Material	Brand	Catalog
Assay Plate (Low-volume White 96-well Microplate)	VKEY-BIO	M2000702N
Assay Plate (Low-volume White 384-well Microplate)	VKEY-BIO	M2000102N
Top sealing film (Fluorescent High-Transparency Microplate Top Seals, Direct Readable)	VKEY-BIO	M1000102N
Microplate Reader with TR-FRET module	TECAN	Infinite® 200 PRO

5. Reagent Preparation

5.1 Reaction System

Components	Volume[*4]	Stock Conc.	Working Conc.	Final Conc.
Test samples or standards	10 μL	\	\	\
Biotin-FcRn & SA-Solar Eu Mix	5 μL	100X	1X	\
Human IgG-LA	5 μL	100X	1X	\

[*4] Recommended Format for low-volume 384-well microplate; For 96-well or 1536-well microplates, proportionally scale the reaction system.

5.2 Reagent Preparation

- Thaw buffers at room temperature. the buffers can be stored at 2-8 ℃.
- Thaw the other reagents on ice and equilibrate to RT before use. Aliquot the stock into single-use volumes (recommended minimum: 10μL) to avoid repeated freeze-thaw cycles. Store these aliquots at -60 ℃ or below.
- Use the provided buffers to prepare samples and detection reagents, to ensure the accuracy and stability of experimental results.
- Prepare and dilute reagents according to the kit technical manual.
- Prepare all reagents immediately before use, unless otherwise specified in the "Working Solution Preparation" section.
- Gently mix the reagents. Avoid Vortex.

6. Working Solution Preparation

6.1 Standard Preparation

- Prepare serially diluted standards as below. Use Diluent Buffer or a solution with the same matrix as the test sample(recommended) to prepare the standard curve. Determine the total volume of standard preparation according to experimental needs, the volumes presented in the table are for reference .

Standard Curve	Working Conc. (ug/ml)	Final Conc. (ug/ml)	Dilution
NC	-	-	See below*[5]
STD-10	2,000.0	1,000.0	30 μL Standard Stock + 30 μL Diluent Buffer
STD-9	500.0	250.0	15 μL STD-10 + 45 μL Diluent Buffer
STD-8	125.0	62.50	15 μL STD-9 + 45 μL Diluent Buffer
STD-7	31.25	15.63	15 μL STD-8 + 45 μL Diluent Buffer
STD-6	7.813	3.906	15 μL STD-7 + 45 μL Diluent Buffer
STD-5	1.953	0.977	15 μL STD-6 + 45 μL Diluent Buffer
STD-4	0.488	0.244	15 μL STD-5 + 45 μL Diluent Buffer
STD-3	0.122	0.061	15 μL STD-4+ 45 μL Diluent Buffer
STD-2	0.031	0.015	15 μL STD-3 + 45 μL Diluent Buffer
STD-1	0.008	0.004	15 μL STD-2 + 45 μL Diluent Buffer
STD-0 (PC)	0.00	0	45 μL Diluent Buffer

*[5] Negative Control (NC): 10 μL Diluent Buffer or solution with the same matrix as the sample + 5 μL Detection Buffer + 5 μL Biotin-FcRn & SA-Solar Eu Mix.

6.2 Sample Preparation

- Dilute the test sample with Diluent Buffer. It is recommended to dilute the test sample 4-fold or more with Diluent Buffer before detection.

6.3 Conjugate Preparation

- **Preparation of Biotin-FcRn & SA-Solar Eu Mix Working Solution (1X):** The stock solution of Biotin-FcRn & SA-Solar Eu Mix is 100X; dilute 1 volume of stock solution with 99 volumes of Detection Buffer.

- **Preparation of Human IgG-LA Working Solution (1X):** The stock solution of Human IgG-LA is 100X, dilute 1 volume of stock solution with 99 volumes of Detection Buffer.

7. Procedure

- Follow the steps in the table below.

	Negative Control	Standard curve	Test samples
Step 1	10 µL Diluent Buffer*[6]	10 µL Serially Diluted Standards	10 µL Prepared Test Sample
Step 2	5 µL Detection Buffer	5 µL Human IgG-LA (1X)	
Step 3	5 µL Biotin-FcRn & SA-Solar Eu Mix		
Step 4	Seal the microplate with Top sealing film to prevent liquid evaporation, Incubation (RT, 25 ℃) for 1h to overnight		
Step 5	Record the data on a TR-FRET compatible microplate reader, No need to remove microplate Top Seals		

*[6] 10 µL Diluent Buffer or solution with the same matrix as the sample.

8. Data Analysis

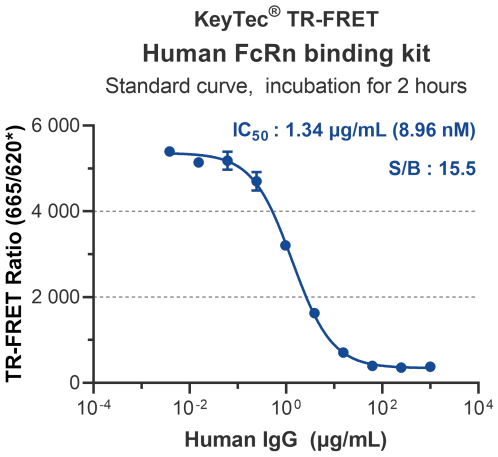
- Calculate the 665 nm/620 nm Ratio (TR-FRET Ratio) and the percentage coefficient of variation (CV %) for each well.

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

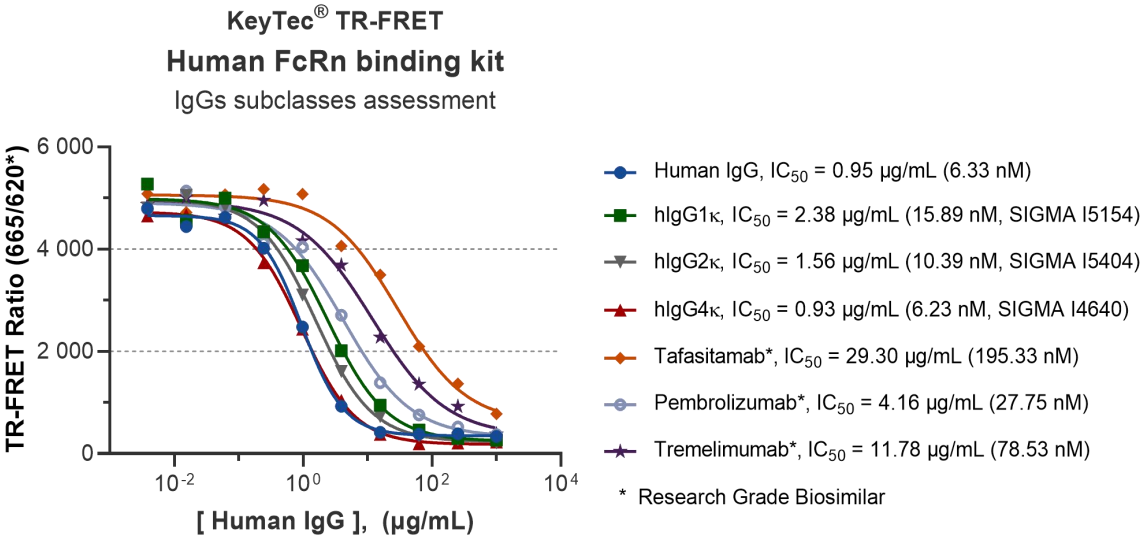
9. Summary

9.1 Standard Curve

Standard	Final Conc. (µg/mL)	Final Conc. (nM)	TR-FRET Ratio	CV%
STD-11	-	-	152	6.3
STD-10	1,000.0	6,666.7	379	3.0
STD-9	250.0	1,666.7	358	1.5
STD-8	62.50	416.7	428	0.0
STD-7	15.63	104.2	706	5.3
STD-6	3.906	26.04	1,628	4.7
STD-5	0.977	6.510	3,206	1.7
STD-4	0.244	1.628	4,702	4.6
STD-3	0.061	0.407	5,182	4.1
STD-2	0.015	0.102	5,073	1.9
STD-1	0.004	0.025	5,399	1.9
STD-0	0	0	5,186	4.6

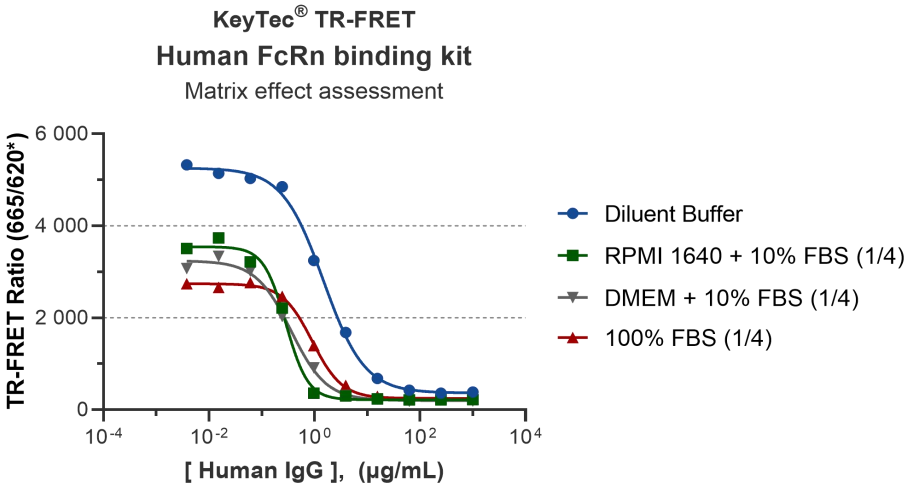


9.2 Binding Affinity of Various IgG Isotypes

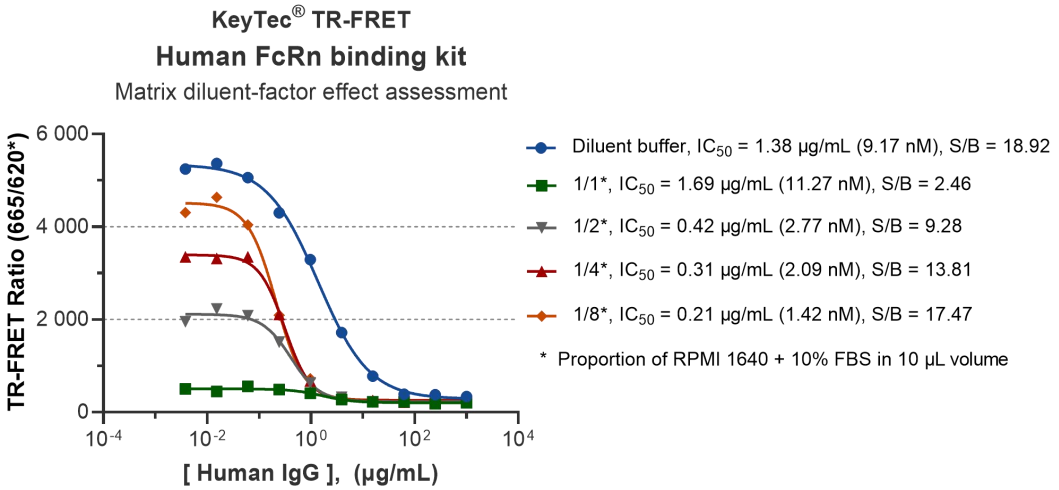


9.3 Assessment of Matrix Effect

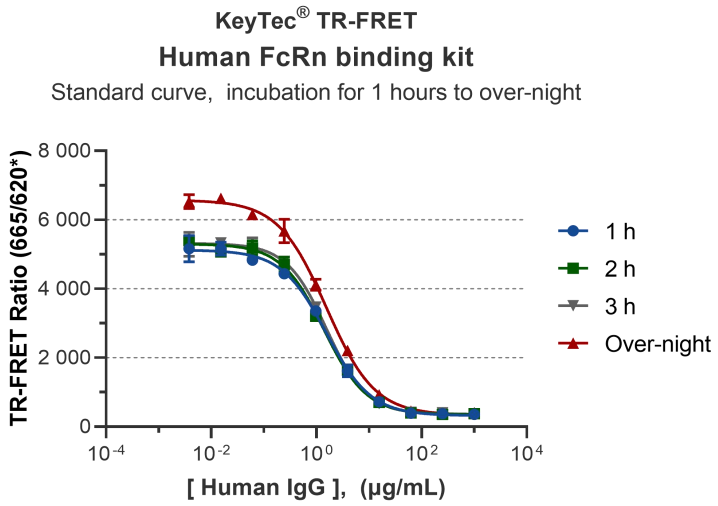
- Dilute standards with different solutions (e.g., Diluent Buffer alone, or a 1:3 mixture of RPMI1640+10%FBS and Diluent Buffer), then determine the S/N ratio and IC_{50} for each condition.



- Dilute standards with the same solutions at different dilution factor (e.g., a 1:1 mixture of RPMI1640+10%FBS and Diluent Buffer), then determine the S/N ratio and IC_{50} for each condition.



9.4 Optimization of Incubation Time



Note: Sample 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