CAT.&Size: A1040015S (500 tests)
A1040015L (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 CD64 (FcγRI) Binding Kit
Technical Manual

1. Introduction

KeyTec® TR-FRET Human CD64 (FcγRI) Binding Kit is designed to screen IgGs that binds to Human CD64 (FcγRI), and provides an ideal solution to assess the ADCC and ADCP of antibody candidate drugs. Human CD64 (FcγRI) is hereinafter referred as CD64. This kit 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.

KeyTec® TR-FRET Solar Eu*1- anti-Tag1 antibody can specifically recognize the CD64 protein with Tag1. The KeyTec® TR-FRET LA*2- Human IgG can bind to the CD64 protein, bringing Solar Eu (referred as donor) and LA (referred as acceptor) close to each other. Under excitation by an external light source, energy resonance transfer occurs between the donor and the acceptor. The binding affinity between CD64 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 CD64, and the TR-FRET signal intensity is inversely proportional to the 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

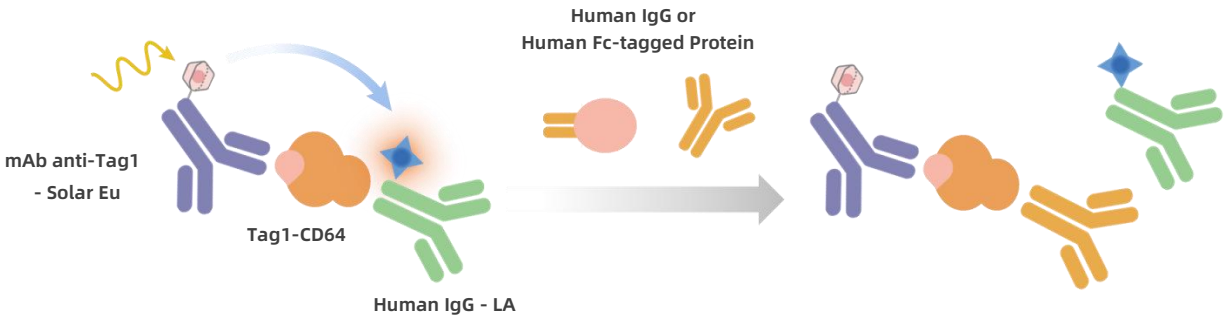


Figure 1. The Principle of KeyTec® TR-FRET Human CD64 Binding Assay

2. Components

Components	Storage	A1040015S (500 tests*3)	A1040015L (5,000 tests*3)
mAb anti-Tag1 - Solar Eu (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
Tag1-CD64 (100X)	≤ -60 ℃	1 vial 25 μL/vial	1 vial 250 μL/vial
Human IgG (4 mg/mL)	≤ -60 ℃	1 vial 120 μL/vial	2 vials 120 μL/vial
Binding Assay Diluent Buffer	2-8 ℃	1 bottle 50 mL/bottle A1010001S	1 bottle 200 mL/bottle A1010001L
Solar Eu Detection Buffer	2-8 ℃	1 bottle 30 mL/bottle A1010002S	1 bottle 120 mL/bottle A1010002L

*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 °C 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	5 μL	\	\	\
Tag1-CD64	5 μL	100X	1X	\
mAb anti-Tag1 - Solar Eu	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 sample 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 only.

Standard Curve	Working Conc. (μg/mL)	Final Conc. (μg/mL)	Dilution
NC	-	-	See below*[5]
STD-10	1,600.0	400.0	20 μL standard stock + 30 μL Diluent Buffer
STD-9	400.0	100.0	10 μL STD-10 + 30 μL Diluent Buffer
STD-8	100.0	25.00	10 μL STD-9 + 30 μL Diluent Buffer
STD-7	25.00	6.250	10 μL STD-8 + 30 μL Diluent Buffer
STD-6	6.250	1.563	10 μL STD-7 + 30 μL Diluent Buffer
STD-5	1.563	0.391	10 μL STD-6 + 30 μL Diluent Buffer
STD-4	0.391	0.098	10 μL STD-5 + 30 μL Diluent Buffer
STD-3	0.098	0.024	10 μL STD-4 + 30 μL Diluent Buffer
STD-2	0.024	0.006	10 μL STD-3 + 30 μL Diluent Buffer
STD-1	0.006	0.002	10 μL STD-2 + 30 μL Diluent Buffer
STD-0 (PC)	0	0	30 μL Diluent Buffer

*[5] Negative Control (NC): 5 μL Diluent Buffer or solution with the same matrix as the sample + 5 μL Diluent Buffer + 5 μL Human IgG - LA + 5 μL 1X mAb anti-Tag1-Solar Eu.

6.2 Sample Preparation

- Dilute the test sample with Diluent Buffer or a solution with the same matrix as the sample.

6.3 Conjugate Preparation

- **Preparation of mAb anti-Tag1 - Solar Eu Working Solution(1X):** The stock solution of mAb anti-Tag1 - Solar Eu is 100X, dilute 1 volume of stock solution with 99 volumes of Detection Buffer.

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

- **Preparation of Premixed Working Solution:** Mix 1X mAb anti-Tag1 - Solar Eu working solution and 1X Human IgG - LA working solution at a ratio of 1:1. The volume of the premixed solution is 10 µL per well. Alternatively, quickly prepare the premixed working solution with reference as below.

Components	Premixed Working conc.	Working Conc.	Stock Conc.	Dilution Factor	Stock µL	Detection Buffer µL	Total Volume µL
mAb anti-Tag1 - Solar Eu	0.5X	1X	100X	200	5	990	1,000
Human IgG - LA	0.5X	1X	100X	200	5		

6.4 Preparation of Protein

- The Tag1-CD64 stock is 100X; dilute 100-fold with Diluent Buffer to obtain 1X working solution, with a volume of 5 µL per well.

7. Procedure

- Follow the steps in the table below.

Operation Steps	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	10 μL Human IgG-LA and mAb anti-Tag1-Solar Eu Premixed Working Solution [*7]		
Step 3	5 μL Diluent Buffer	5 μL Tag1-CD64 (1X)	
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] 5 μL Diluent Buffer or solution with the same matrix as the sample.

*[7] It is recommended to use the addition mode of premixed detection antibody pairs, which can not only reduce operation steps but also reduce deviations introduced by operations.

8. Data Analysis

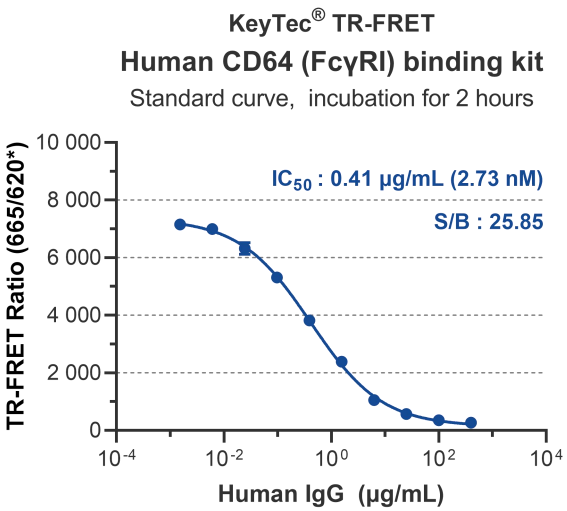
- Calculate the 665 nm/620 nm Ratio (TR-FRET Ratio) and the percentage coefficient of variation (CV %) for each well.

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

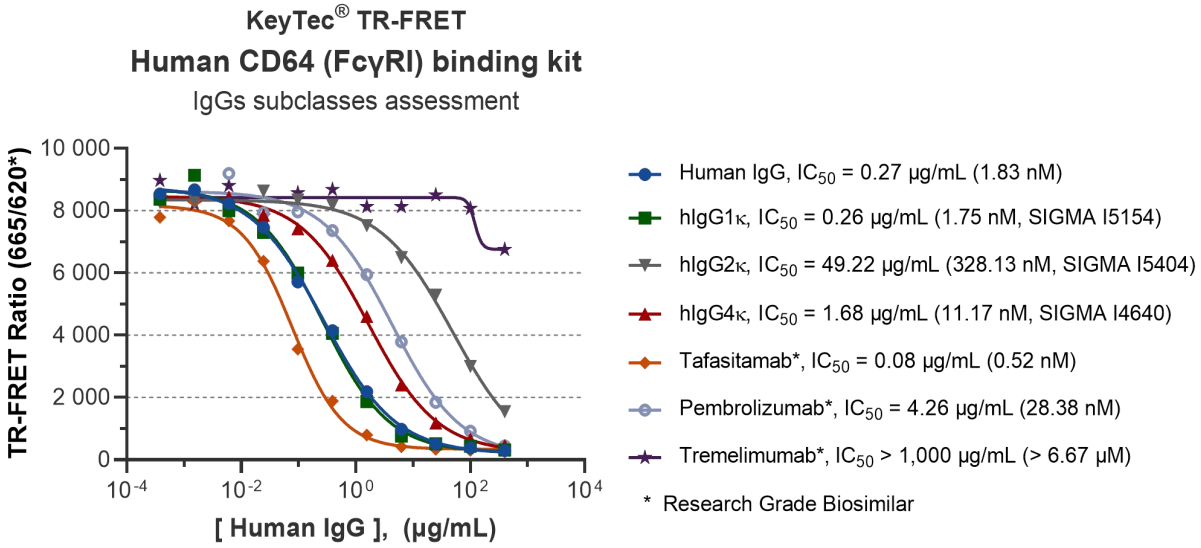
9. Summary

9.1 Standard Curve

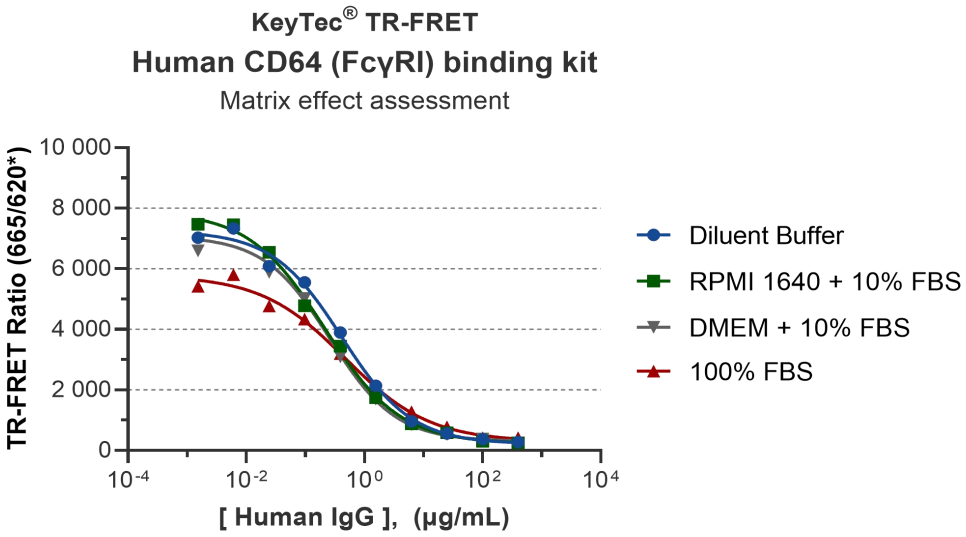
Standard	Final Conc. (μg/mL)	Final Conc. (nM)	TR-FRET Ratio	CV %
NC	\	\	258	2.4
STD-10	400.0	2,666.7	268	4.2
STD-9	100.0	666.7	357	5.0
STD-8	25.00	166.7	570	2.0
STD-7	6.250	41.67	1,055	3.1
STD-6	1.563	10.42	2,390	2.4
STD-5	0.391	2.604	3,824	0.0
STD-4	0.098	0.651	5,311	0.8
STD-3	0.024	0.163	6,320	3.2
STD-2	0.006	0.041	6,998	1.2
STD-1	0.002	0.010	7,157	1.5
STD-0	0	0	6,683	4.4



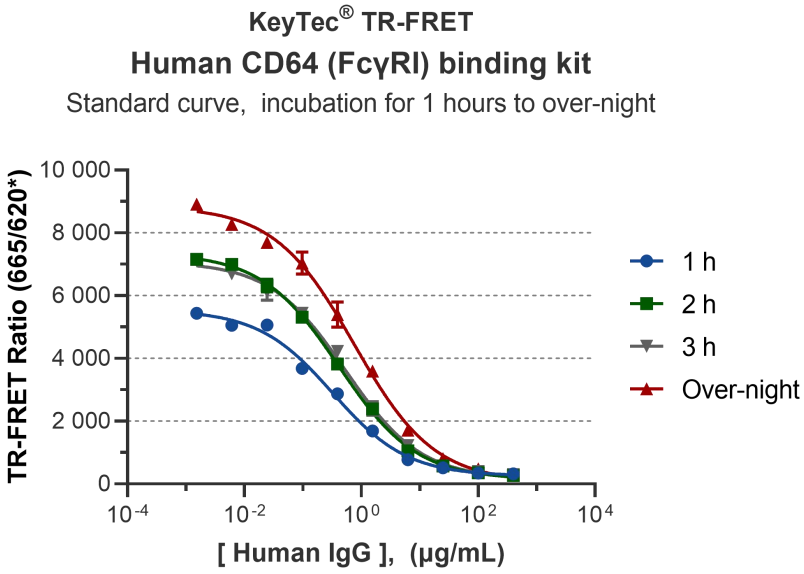
9.2 Binding Affinity of Various IgG Subtypes



9.3 Assessment of Matrix Effect



9.4 Test Results in Different 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