

Human FcRn binding Kit (TR-FRET)

Pack Size: 100 Tests & 500 Tests

Catalog Number: FRT-01

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

INTENDED USE

This kit is designed to facilitate the half-life evaluation of antibody drug candidates, and also high-throughput screening of FcRn inhibitors. It can also be used as a universal detection tool to identifying the ability of antibody drugs to bind to FcRn. It is for research use only (RUO).

BACKGROUND

FcRn (neonatal Fc receptor for IgG) is a heterodimer comprising a β 2-microglobulin (β 2m) light chain and a major histocompatibility complex class I-like heavy chain. It is widely accepted that the interaction of IgGs with the Fc receptor (FcRn) plays a critical role in regulating IgG homeostasis in vivo. FcRn interacts with the CH2-CH3 portion of the Fc domain of IgGs in a tightly regulated pH-dependent manner with high affinity binding occurring at an acidic (pH=6) and weak to no binding interactions as the pH is raised to neutral (7.4). FcRn is responsible for the extended serum half-life of IgG and also serum albumin, and for the transport across endothelial and epithelial barriers, increasing the overall bioavailability of IgG and serum albumin. The binding affinity between FcRn and IgG are commonly used to characterize the metabolic levels of antibody drugs.

The Human FcRn Binding Kit (TR-FRET) takes advantage of binding of Europium-chelate labeled FcRn (donor) and FA labeled Human IgG1 antibody (acceptor) in a homogeneous (no wash) TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer) competition assay to measure the interaction between human FcRn and antibody drug candidates or FcRn inhibitors. It is designed to facilitate the half-life evaluation of antibody drug candidates, and also high-throughput screening of FcRn inhibitors within 0.5-1 hours. It is highly sensitive, short detection time and easy to use.

PRINCIPLE OF THE ASSAY

This Human FcRn binding Kit (TR-FRET) is based on TR-FRET technology (Time-Resolved Fluorescence Resonance Energy Transfer). Use the mixture of biotinylated FcRn and Europium-chelate labeled streptavidin as the donor, FA labeled Human IgG1 antibody as the acceptor.

Your experiment will include 3 simple steps:

- 1) Mix the sample or Human IgG standard in the kit with Human FcRn & β 2m Heterodimer Protein Europium-chelate (Donor) and incubate at room temperature for 0.5 hour.

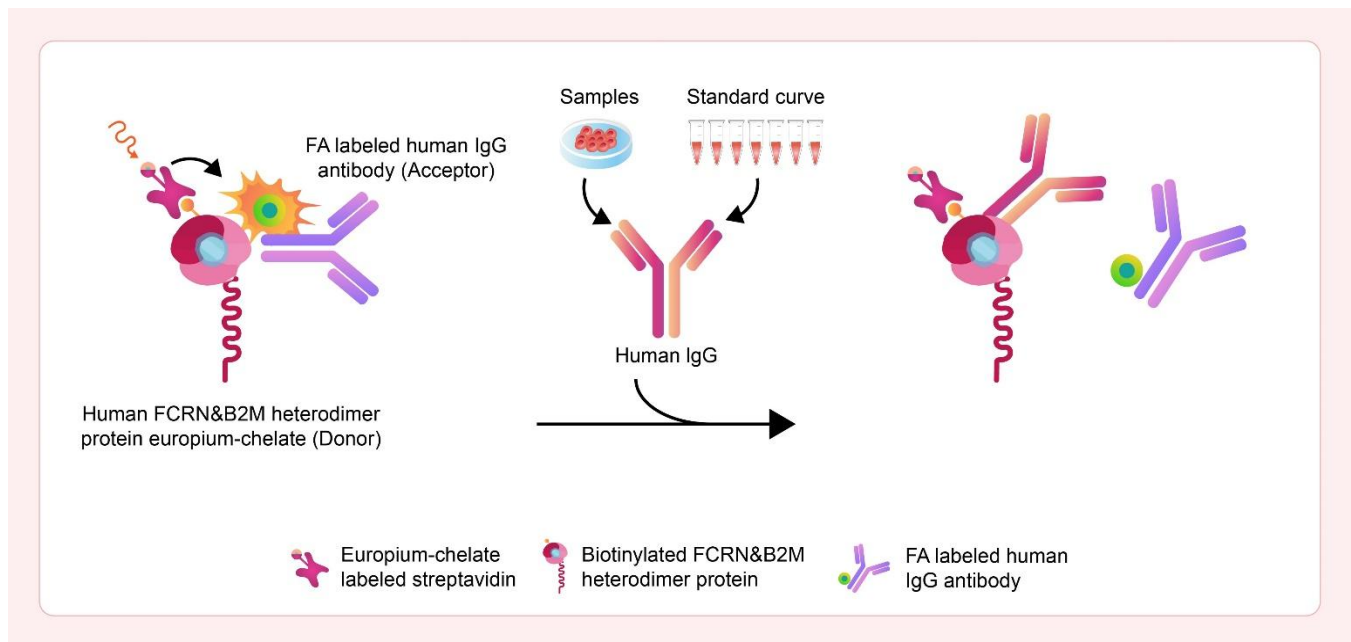
2) Add FA labeled human IgG antibody (Acceptor) and incubate at room temperature for more than 0.5 hour.

3) Use the TR-FRET module of a microplate reader to read the fluorescence signal at 665 nm and 620 nm. Calculate Ratio based on the formula $\text{Ratio} = \frac{\text{Signal } 665 \text{ nm}}{\text{Signal } 620 \text{ nm}} \times 10^4$. The Ratio value is negatively correlated with the antibody content in the sample.

— When the sample does not contain FcRn binding components, the donor and acceptor are in close proximity because of the binding of FcRn and FA labeled Human IgG1 antibody. The 620 nm signal emitted by the donor under specific light source excitation is received by the acceptor, emitting a 665 nm signal.

— When the sample contains FcRn binding components, the components inhibit the binding between the donor and acceptor and thereby prevents FRET from occurring.

FIG.1 PRINCIPLE OF THE ASSAY



MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (100 Tests)	Size (500 Tests)	Format	Storage	
					Unopened	Opened
FRT01-C01	Human FCRN&B2M Heterodimer Protein Europium-chelate	2.7 µg	13.5 µg	Powder	2-8°C, avoid light	-70°C, avoid light
FRT01-C02	FA labeled human IgG antibody	2.4 µg	12 µg	Powder	2-8°C, avoid light	-70°C, avoid light
FRT01-C03	Human IgG standard	200 µg	1 mg	Powder	2-8°C	-70°C
FRT01-C04	Sample Dilution Buffer	10 mL	10 mL	Liquid	2-8°C	2-8°C
FRT01-C05	Detection Buffer	10 mL	10 mL	Liquid	2-8°C	2-8°C

MATERIALS REQUIRED BUT NOT PROVIDED

1. Single channel or multichannel pipettes with 10 µL, 200 µL and 1000 µL precision;
2. 10 µL, 200 µL and 1000 µL pipette tips;
3. Microporous plate shaker;
4. Microplate reader with TR-FRET module which can detect signals at 665 nm/620 nm;
5. Test Tubes;
6. Timer;
7. White plate (96 or 384-well low volume white plate);
8. Deionized or distilled water for reconstitute;

STORAGE AND VALIDITY INSTRUCTIONS

1. Unopened kit should be stored at 2°C-8°C upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20°C-25°C) before use.

2. Reconstitute the provided lyophilized materials to stock solutions with water as recommended in Tab.2 and solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortexing. The reconstituted stock solutions should be stored at -70°C. It is recommended not to freeze-thaw more than 2 times.

Note: Human FCRN&B2M Heterodimer Protein Europium-chelate and FA labeled human IgG antibody stock solution should be protected from light.

TABLE 2. RECONSTITUTION METHODS FOR 100 TESTS AND 500TESTS

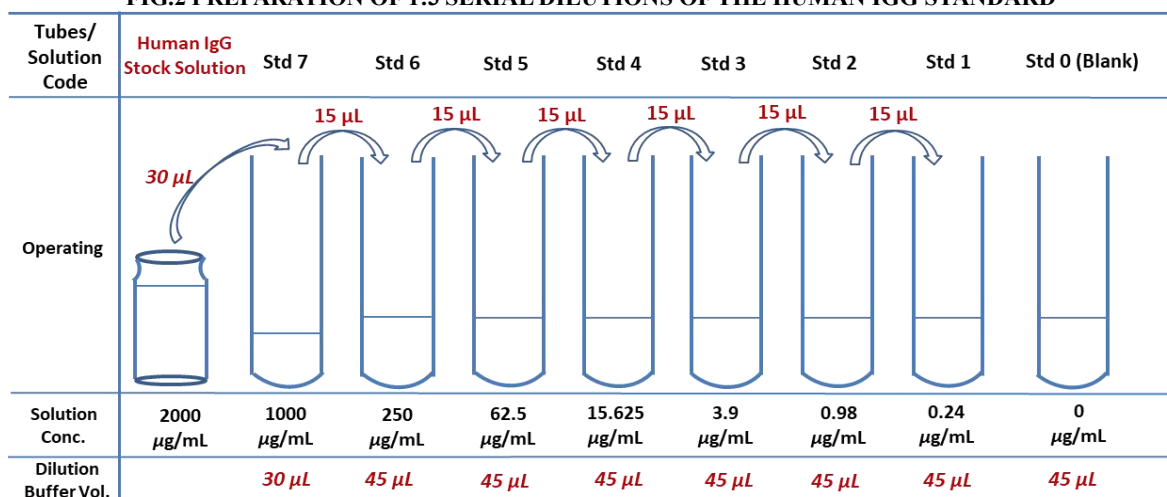
Catalog	Components	Stock Solution Conc.	Size(100 Tests)		Size(500 Tests)	
			Amount	Reconstitution Buffer and Vol.	Amount	Reconstitution Buffer and Vol.
FRT01-C01	Human FCRN&B2M Heterodimer Protein Europium-chelate	45 µg/mL	2.7 µg	60 µL water	13.5 µg	300 µL water
FRT01-C02	FA labeled human IgG antibody	40 µg/mL	2.4 µg	60 µL water	12 µg	300 µL water
FRT01-C03	Human IgG standard	2000 µg/mL	200 µg	100 µL water	1 mg	500 µL water

RECOMMENDED PROTOCOL

1. Add Samples

- 1.1 Make series dilution of the samples as appropriate.
- 1.2 If you intend to use the provided Human IgG standard as a reference (Std.), you may dilute the antibody as recommend in FIG. 2 Dilute the sample to be tested appropriately using a Sample Dilution Buffer.
- 1.3 Add 10 µL of sample and standard solution to each well according to our recommendation (FIG. 3) or your own plate setup.

FIG.2 PREPARATION OF 1:3 SERIAL DILUTIONS OF THE HUMAN IGG STANDARD



2. Add Donor

Dilute **Human FCRN&B2M Heterodimer Protein Europium-chelate** stock solution (45 µg/mL) to 4.5 µg/mL with **Detection Buffer** to make **Donor** working solution. The working solution should be prepared immediately before use and should not be stored. Add 5 µL of Donor working solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (20°C-25°C) for 0.5 hour on orbital shaker at 400-600 rpm to make sure the samples and donor can react adequately.

3. Add Acceptor

Dilute **FA labeled human IgG antibody** stock solution (40 µg/mL) to 4 µg/mL with **Detection Buffer** to make **Acceptor** working solution. The working solution should be prepared immediately before use and should not be stored. Add 5 µL of **Acceptor** working solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (20°C-25°C) for 0.5 hour on orbital shaker at 400-600 rpm.

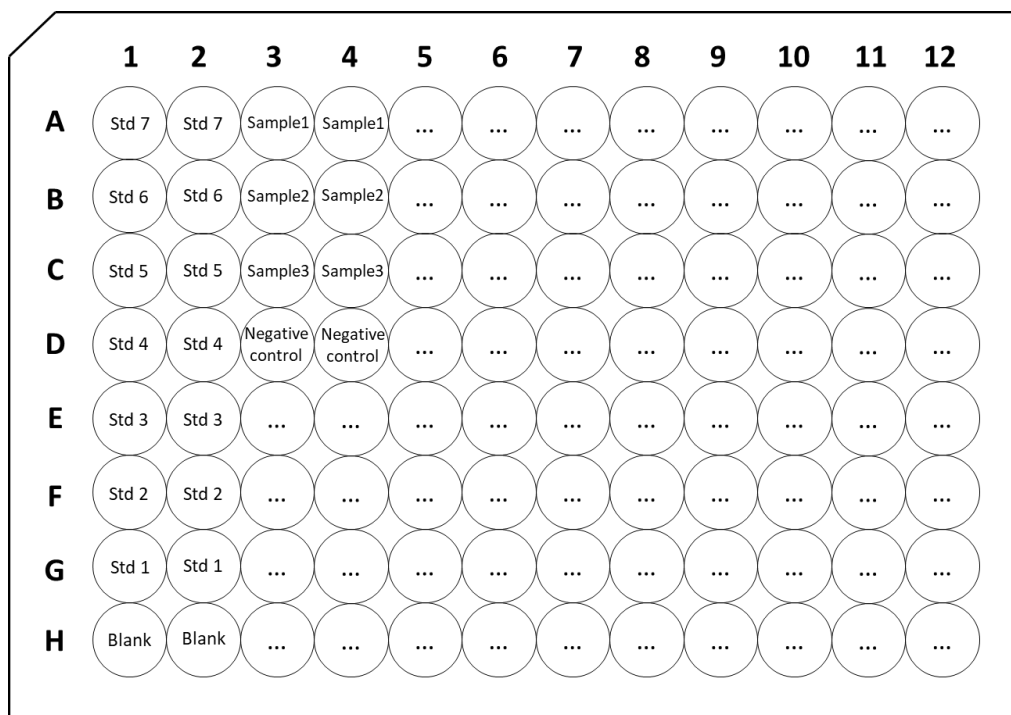
Refer to FIG. 3 and Table 3 for the design of microplate layout according to the experimental requirements, and add the corresponding reaction solution into the corresponding plate holes.

TABLE 3. SAMPLES ADDING TO MICROPLATE

	1	2	3	4
A	10 µL Std7 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Std7 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Sample1 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Sample1 5 µL Donor working solution 5 µL Acceptor working solution
B	10 µL Std6 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Std6 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Sample2 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Sample2 5 µL Donor working solution 5 µL Acceptor working solution
C	10 µL Std5 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Std5 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Sample3 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Sample3 5 µL Donor working solution 5 µL Acceptor working solution
D	10 µL Std4 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Std4 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Sample Dilution Buffer 5 µL Donor working solution 5 µL Detection Buffer	10 µL Sample Dilution Buffer 5 µL Donor working solution 5 µL Detection Buffer
E	10 µL Std3 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Std3 5 µL Donor working solution 5 µL Acceptor working solution
F	10 µL Std2	10 µL Std2

	5 µL Donor working solution 5 µL Acceptor working solution	5 µL Donor working solution 5 µL Acceptor working solution		
G	10 µL Std1 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Std1 5 µL Donor working solution 5 µL Acceptor working solution
H	10 µL Sample Dilution Buffer 5 µL Donor working solution 5 µL Acceptor working solution	10 µL Sample Dilution Buffer 5 µL Donor working solution 5 µL Acceptor working solution

FIG.3 PLATE LAYOUT



4. Data Recording

Use the TR-FRET module of a microplate reader to read the fluorescence signal at 665 nm and 620 nm.

5. Calculate Ratio

Calculate Ratio based on the formula $\text{Ratio} = \frac{\text{Signal } 665 \text{ nm}}{\text{Signal } 620 \text{ nm}} \times 10^4$.

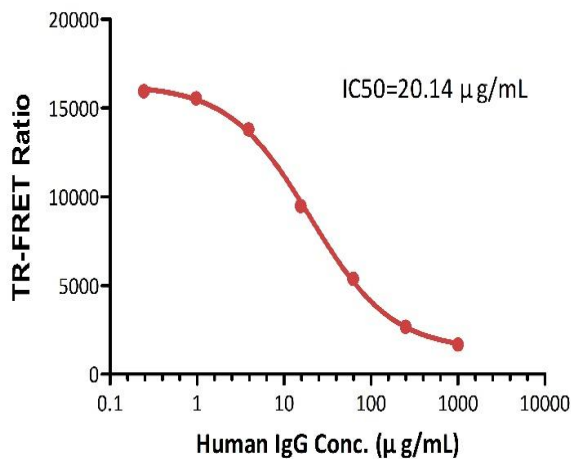
PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.

3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
5. This kit should be stored at 2°C -8°C.
6. Please prepare the working solution of each component according to the needs of the experiment. All prepared working solution is for one-time use and cannot be stored.

TYPICAL DATA

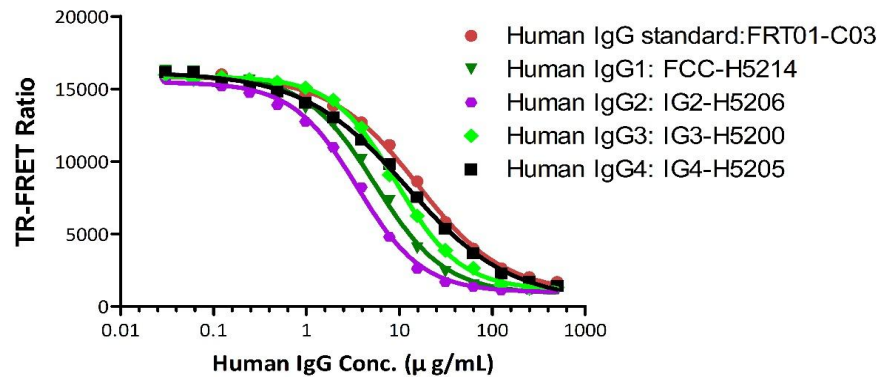
For each experiment, a standard curve needs to be set for each micro-plate, and the specific Ratio value may vary depending on different laboratories, testers, or equipment. Different microplate reader and different gain value may give different fluorescence signal. Please adjust parameters according to the equipment manual. Reduce the gain value when the signal is too high. The following data is from the BMG Labtech Clariostar Plus. This following data is for reference only.



Human IgG standard Conc.	Signal 665 nm	Signal 620 nm	Ratio
1000 µg/mL	3488	20864	1672
250 µg/mL	5713	21352	2676
62.5 µg/mL	10745	19997	5373
15.625 µg/mL	17787	18770	9476
3.9 µg/mL	25657	18594	13799
0.98 µg/mL	28226	18167	15537
0.24 µg/mL	28672	17991	15937
0 µg/mL	29559	18091	16339

DIFFERENT ANTIBODY SUBTYPES DATA

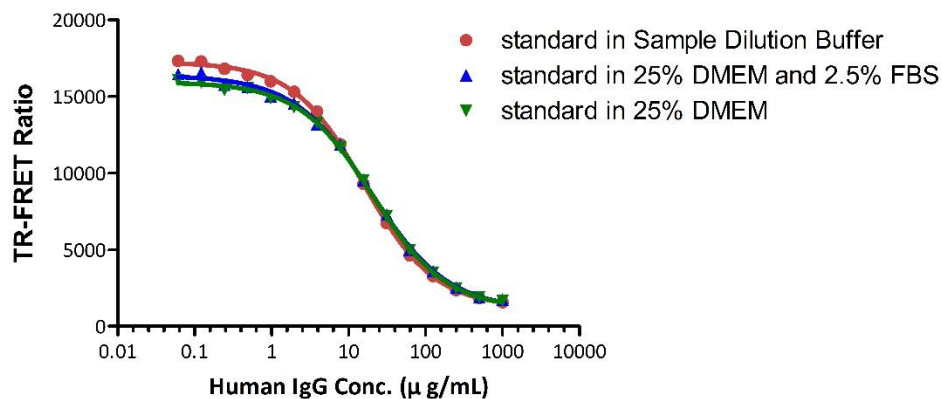
The kit has been used to detect different human IgG isoforms of Human IgG1, Human IgG2, Human IgG3, Human IgG4 with different affinities binding to FcRn. The result is consistent with SPR test results.



Antibody	SPR Affinity with FcRn	IC50 (µg/mL)-TR-FRET	IC50 (nM)-TR-FRET
Human IgG standard (FRT01-C03)	NA	15.7 µg/mL	104.67 nM
Human IgG1 Fc, Tag Free (Cat.No. FCC-H5214)	957 nM	5.504 µg/mL	210.88 nM
Human IgG2 Fc, Tag Free (Cat.No. IG2-H5206)	33.3 nM	3.542 µg/mL	140.56 nM
Human IgG3 Fc, Tag Free (Cat.No. IG3-H5200)	683 nM	9.376 µg/mL	300.42 nM
Human IgG4 Fc, Tag Free (Cat.No. IG4-H5205)	715 nM	12.01 µg/mL	465.50 nM

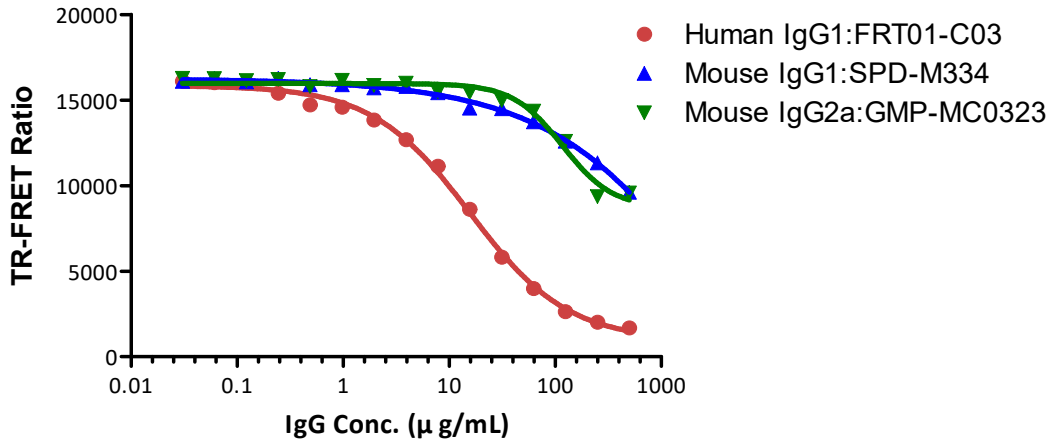
MATRIX EFFECT

Verify potential matrix effects by adding different levels of DEME and FBS to the Sample Diluted buffer.



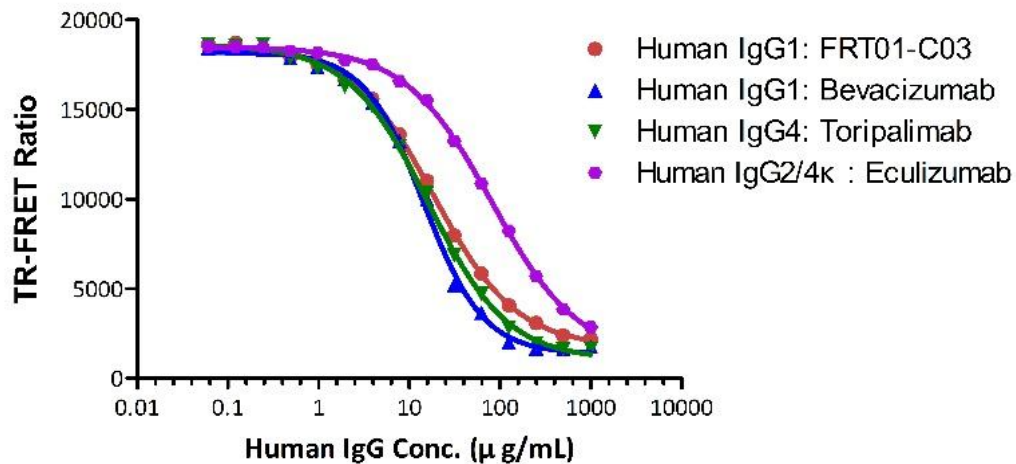
SPECIES SELECTIVITY

The kit is human IgG specific and not compatible with mouse IgG1 and mouse IgG2a.

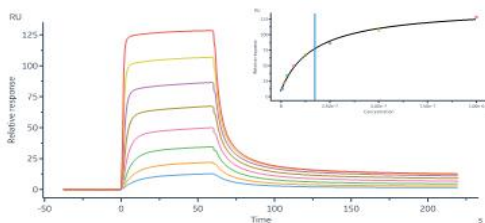


APPLICATION OF FDA APPROVED ANTIBODY DRUGS DETECTION

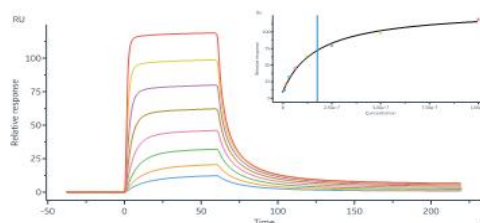
The half-lives of monoclonal antibodies currently in clinical use generally correlate with measurements of the affinity of their Fc for FcRn. The kit has been used to detect three FDA approved antibody drugs with different affinities binding to FcRn. The result is consistent with SPR test results. In the same experiment, the lower the IC50, the stronger binding affinity between IgG antibodies and FcRn, and the longer half-life of the antibody drug. Bevacizumab and Toripalimab have consistent binding affinity with FcRn and have a half-life of about 20 days. The Fc of Eculizumab has been modified to bind relatively weakly to FcRn, resulting in a relatively short half-life of approximately 11 days.



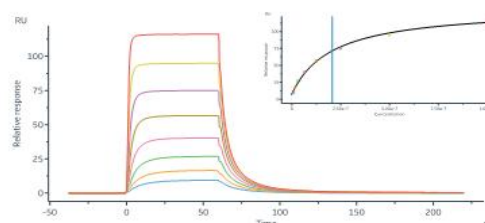
FRT01-C03



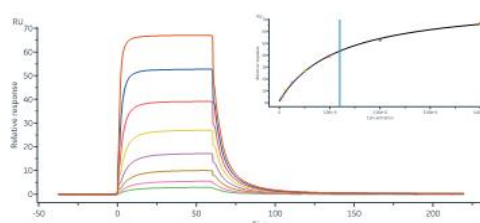
Bevacizumab



Toripalimab



Eculizumab



Antibody	Molecular Weight	IC50 (µg/mL)-TR-FRET	IC50 (nM)-TR-FRET	KD (M)-SPR	Half-life
Human IgG standard (FRT01-C03)	150 kDa	18.66 µg/mL	124.4 nM	1.73E-07	NA
Bevacizumab (Human IgG1)	149 kDa	14.47 µg/mL	97.1 nM	1.76E-07	20 days (11 to 50 days).
Toripalimab (Human IgG4)	150 kDa	16.16 µg/mL	107.7 nM	2.07E-07	10 ± 1.5 days after the first dose and 18 ± 9.4 days at steady state.
Eculizumab	148 kDa	81.39 µg/mL	549.9 nM	1.20E-06	270 h to 414 h(11 to 18 days)

APPLICATION OF HIGH-THROUGHPUT SCREENING OF FcRn INHIBITORS

The kit has been used to detect the Efgartigimod drug and Human IgG1 Fc (C103S, M135Y, S137T, T139E, H316K, N317F), His Tag (Cat.No. IG1-H52H8) that inhibit FcRn.

