FRT-Q034-EN.01

High Performance Human Fc Detection Kit (TR-FRET)

Pack Size: 100 tests & 500 tests

Catalog Number： FRT-Q034

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

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

PRODUCT OVERVIEW

This High Performance Human Fc Detection Kit (TR-FRET) is based on a homogeneous (no wash) competition TR-FRET technology (Time-Resolved Fluorescence Resonance Energy Transfer) to quantify human IgG or human Fc-tagged proteins within one hour. The kit is compatible with both natural and recombinant human IgG.

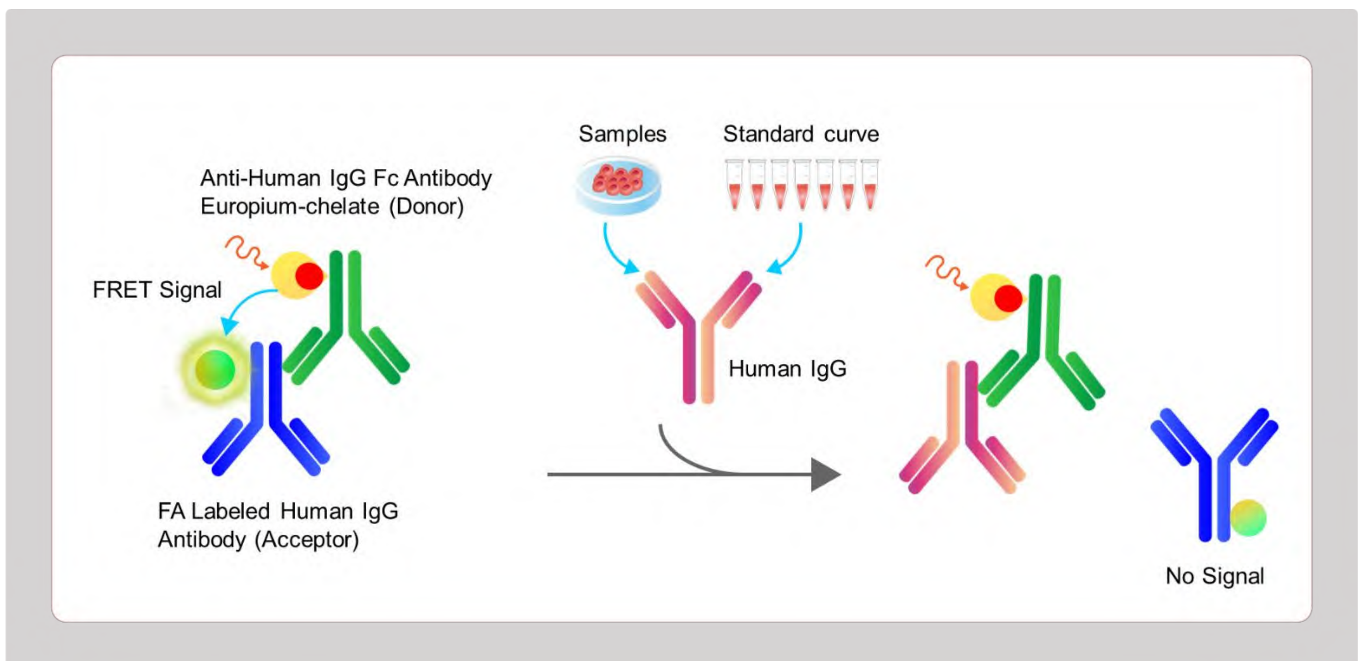
PRINCIPLE OF THE ASSAY

This High Performance Human Fc Detection Kit (TR-FRET) is based on TR-FRET technology (Time-Resolved Fluorescence Resonance Energy Transfer). This assay uses Anti-Human IgG Fc Antibody Europium-chelate as the Donor and FA Labeled Human IgG Antibody as the Acceptor.

— When the sample does not contain human IgG or human Fc-tagged proteins, the Donor and Acceptor are in close proximity because of the binding of Anti-Human IgG Fc Antibody Europium-chelate and FA Labeled Human IgG Antibody. Upon Donor excitation with light of a specific wavelength (337 nm), in addition to Donor emission (620 nm), non-radiative transfer of energy occurs between Donor and Acceptor, resulting in Acceptor emission (665 nm).

— When the sample contains human IgG or human Fc-tagged proteins, the components inhibit the binding between the Donor and Acceptor and thereby prevent FRET from occurring.

FIGURE 1. PRINCIPLE OF THE ASSAY



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MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (100 tests)	Size (500 tests)	Format	Storage	
					Unopened	Opened
FRTQ034-C01	Anti-Human IgG Fc Antibody Europium-chelate	100 tests	500 tests	Powder	2-8℃, protected from light	-70℃, protected from light
FRTQ034-C02	FA Labeled Human IgG Antibody	100 tests	500 tests	Powder	2-8℃, protected from light	-70℃, protected from light
FRTQ034-C03	Human IgG Standard	400 μg	2 mg	Powder	2-8℃	-70℃
DB-04	TR-FRET Sample Dilution Buffer, pH7.4	50 mL	50 mL	Liquid	2-8℃	2-8℃
DB-05	TR-FRET Detection Buffer, pH7.4	50 mL	50 mL	Liquid	2-8℃	2-8℃

STORAGE AND VALIDITY INSTRUCTIONS

1. Store the unopened kit at 2–8 °C upon receipt.

2. Locate the expiration date on the outer packaging and do not use reagents beyond their expiration date.

3. The opened kit should be stored according to the storage conditions listed in the Components Table. The shelf life of the opened kit is 30 days from the date of opening.

MATERIALS REQUIRED BUT NOT PROVIDED

TABLE 2. MATERIALS REQUIRED BUT NOT PROVIDED

Items	Specifications	Recommendation
Single channel and multi-channel pipettes	Must be calibrated pipettes, with 10 µL, 200 µL and 1000 µL precision	Different pipettes have different levels of precision. Please choose pipettes with appropriate precision.
Pipette tips	Low adsorption pipette tips, all tips need to fit the pipettes	-
96 or 384-well white plate	Non-transparent 96- or 384-well low-volume white plates typically provide the lowest background signal.	For example, 384-well white plate (iSTAR, Cat. No. GT247.008)

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Microplate shaker	For plate shaking	-
EP tubes	For dilution of samples	-
Microplate reader	Plate reader capable of measuring signals at 665 nm/620 nm in TR-FRET mode	For example, BMG LABTECH CLARIOstar® Plus; TECAN Spark®, Infinite® F Nano^{+}, Infinite® F Plex
Timer	-	-
Deionized or distilled water	For reconstitution	-

REAGENT PREPARATION

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

2. Reconstitute the lyophilized materials with deionized or distilled water as described in Table 3 to prepare stock solutions. Allow the solutions to solubilize for 15-30 minutes at room temperature with occasional gentle mixing by inverting the tube 2-3 times. Avoid vigorous shaking or vortexing. The reconstituted stock solutions should be stored at –70°C. It is recommended not to freeze–thaw more than twice.

Note: Both the Anti-Human IgG Fc Antibody Europium-chelate and the FA Labeled Human IgG Antibody stock solutions are light-sensitive and should be protected from light.

TABLE 3. RECONSTITUTION METHODS FOR 100 TESTS AND 500 TESTS

Catalog	Components	Size (100 tests)		Size (500 tests)		Stock Solution Conc.
		Amount	Reconstitution Buffer and Vol.	Amount	Reconstitution Buffer and Vol.	
FRTQ034-C01	Anti-Human IgG Fc Antibody Europium-chelate	100 tests	90 μL water	500 tests	450 μL water	/
FRTQ034-C02	FA Labeled Human IgG Antibody	100 tests	90 μL water	500 tests	450 μL water	/
FRTQ034-C03	Human IgG Standard	400 μg	200 μL water	2 mg	1000 μL water	2000 μg/mL

RECOMMENDED PROTOCOL

1. Preparation of Sample

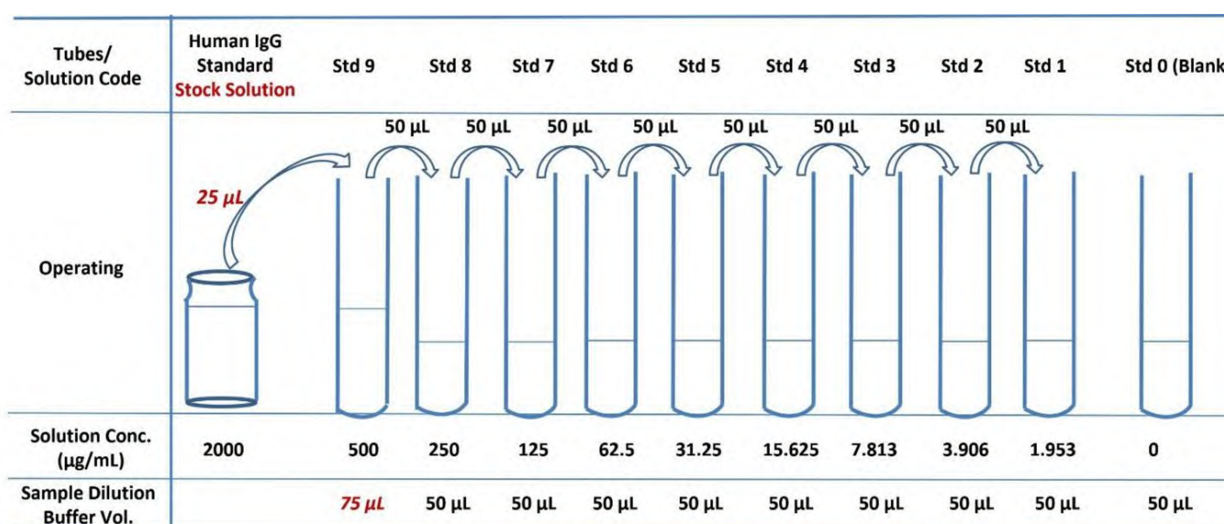
1.1 Dilute sample appropriately using TR-FRET Sample Dilution Buffer, pH7.4 (DB-04) or a solution with the same matrix as the test samples. Samples with a concentration above the highest standard (Std 9: 500 μg/mL) must be

diluted.

1.2 Serially dilute the provided Human IgG Standard (FRTQ034-C03) using TR-FRET Sample Dilution Buffer, pH7.4 (DB-04) or a solution with the same matrix as the test samples as described in Figure 2.

Note: It is recommended to prepare the standard curve using a solution with the same matrix as the test samples, so as to reduce interference from matrix effects and obtain more accurate back-calculated sample concentrations. Do not use TR-FRET Detection Buffer, pH7.4 (DB-05) or any buffers from external sources.

FIGURE 2. PREPARATION OF SERIAL DILUTIONS OF THE HUMAN IGG STANDARD



2. Preparation of Donor working solution

Dilute the **Anti-Human IgG Fc Antibody Europium-chelate (FRTQ034-C01)** stock solution 10-fold with **TR-FRET Detection Buffer, pH7.4 (DB-05)** to prepare the Donor working solution. The working solution should be prepared immediately before use and must not be stored.

Note: Do not use the TR-FRET Sample Dilution Buffer, pH7.4 (DB-04), or any other buffers from external sources as substitutes, as doing so could result in unexpected or inconsistent experimental results.

3. Addition of Samples and Donor reagent

Add 5 µL of diluted samples and serially diluted standards to the designated wells according to our recommendation (Table 4) or your own plate layout. Then add 7.5 µL of the Donor working solution to each well. Seal the plate with a microplate sealing film and incubate at room temperature (20°C-25°C) for 30 minutes on an orbital shaker at 400-600

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rpm to ensure the samples and Donor can react adequately.

4. Preparation of Acceptor working solution

Dilute the **FA Labeled Human IgG Antibody (FRTQ034-C02)** stock solution 10-fold with **TR-FRET Detection Buffer, pH7.4 (DB-05)** to prepare the Acceptor working solution. The working solution should be prepared immediately before use and must not be stored.

Note: The Detection Buffer of Acceptor is the same as Donor.

5. Addition of Acceptor reagent

Add 7.5 μL of Acceptor working solution to each well. Seal the plate with a microplate sealing film and incubate at room temperature (20°C-25°C) for 30 minutes on orbital shaker at 400-600 rpm.

Refer to Figure 3 and Table 4 for the recommended microplate layout. Add the corresponding reaction solutions to the designated wells according to the experimental requirements.

TABLE 4. SAMPLES ADDING TO MICROPLATE

	1	2	3	4
A	5 μL Std 9 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Std 9 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Std 1 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Std 1 7.5 μL Donor working solution 7.5 μL Acceptor working solution
B	5 μL Std 8 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Std 8 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample Dilution Buffer 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample Dilution Buffer 7.5 μL Donor working solution 7.5 μL Acceptor working solution
C	5 μL Std 7 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Std 7 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample1 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample1 7.5 μL Donor working solution 7.5 μL Acceptor working solution
D	5 μL Std 6 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Std 6 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample2 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample2 7.5 μL Donor working solution 7.5 μL Acceptor working solution
E	5 μL Std 5 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Std 5 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample… 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample… 7.5 μL Donor working solution 7.5 μL Acceptor working solution
F	5 μL Std 4 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Std 4 7.5 μL Donor working solution 7.5 μL Acceptor working solution	5 μL Sample Dilution Buffer 7.5 μL Donor working solution 7.5 μL Detection Buffer	5 μL Sample Dilution Buffer 7.5 μL Donor working solution 7.5 μL Detection Buffer

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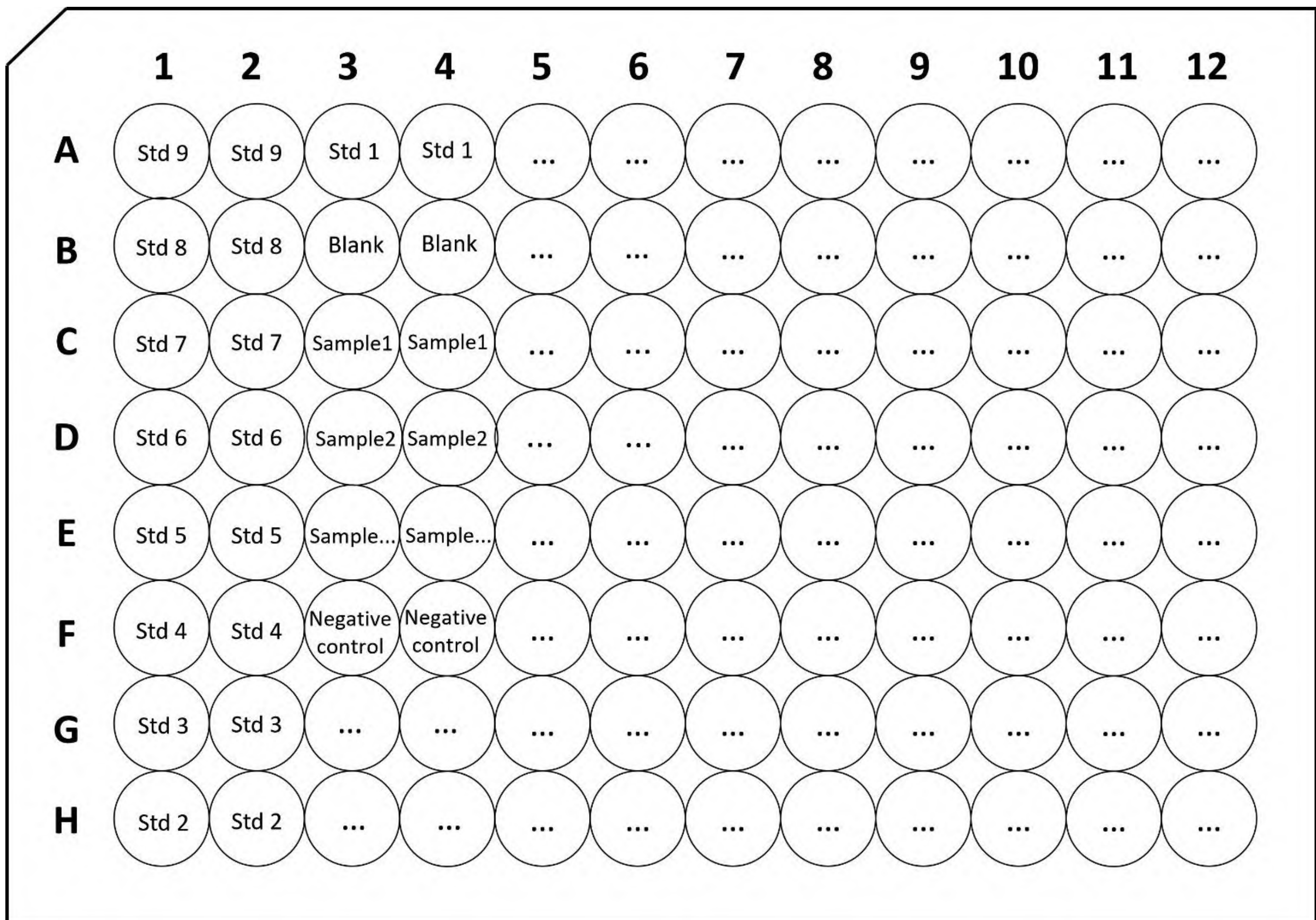
G	5 µL Std 3 7.5 µL Donor working solution 7.5 µL Acceptor working solution	5 µL Std 3 7.5 µL Donor working solution 7.5 µL Acceptor working solution		
H	5 µL Std 2 7.5 µL Donor working solution 7.5 µL Acceptor working solution	5 µL Std 2 7.5 µL Donor working solution 7.5 µL Acceptor working solution		

FIGURE 3. PLATE LAYOUT



5. Plate Reading

Use the TR-FRET module of a microplate reader to measure the fluorescence signals at 665 nm and 620 nm (the excitation wavelength is 337 nm).

CALCULATION OF RESULTS

1. For each individual well, calculate the ratio of the Acceptor (665nm) and Donor (620nm) emission signals using the formula below:

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4.$$

2. Calculate the Mean Ratio for each sample, standard or control.

3. The standard curve is plotted with the standard concentration as x-axis and the Ratio as y-axis. Establish a standard curve by processing the data using computer software capable of executing a **four-parameter logistic (4-PL)** curve fitting. Normal range of Standard curve: $R^2 \geqslant 0.9900$.

4. Detection range: 1.953 μg/mL-500 μg/mL. In this assay, the Ratio value is inversely proportional to the analyte concentration. If the Ratio of a test sample is lower than the Ratio corresponding to the 500 μg/mL standard, the analyte concentration of the sample is above the upper limit of quantification. The sample should be diluted with the dilution buffer and re-assayed. If the Ratio of a test sample is higher than the Ratio corresponding to the 1.953 μg/mL standard, the analyte concentration is below the lower limit of quantification and should be reported as ＜1.953 μg/mL.

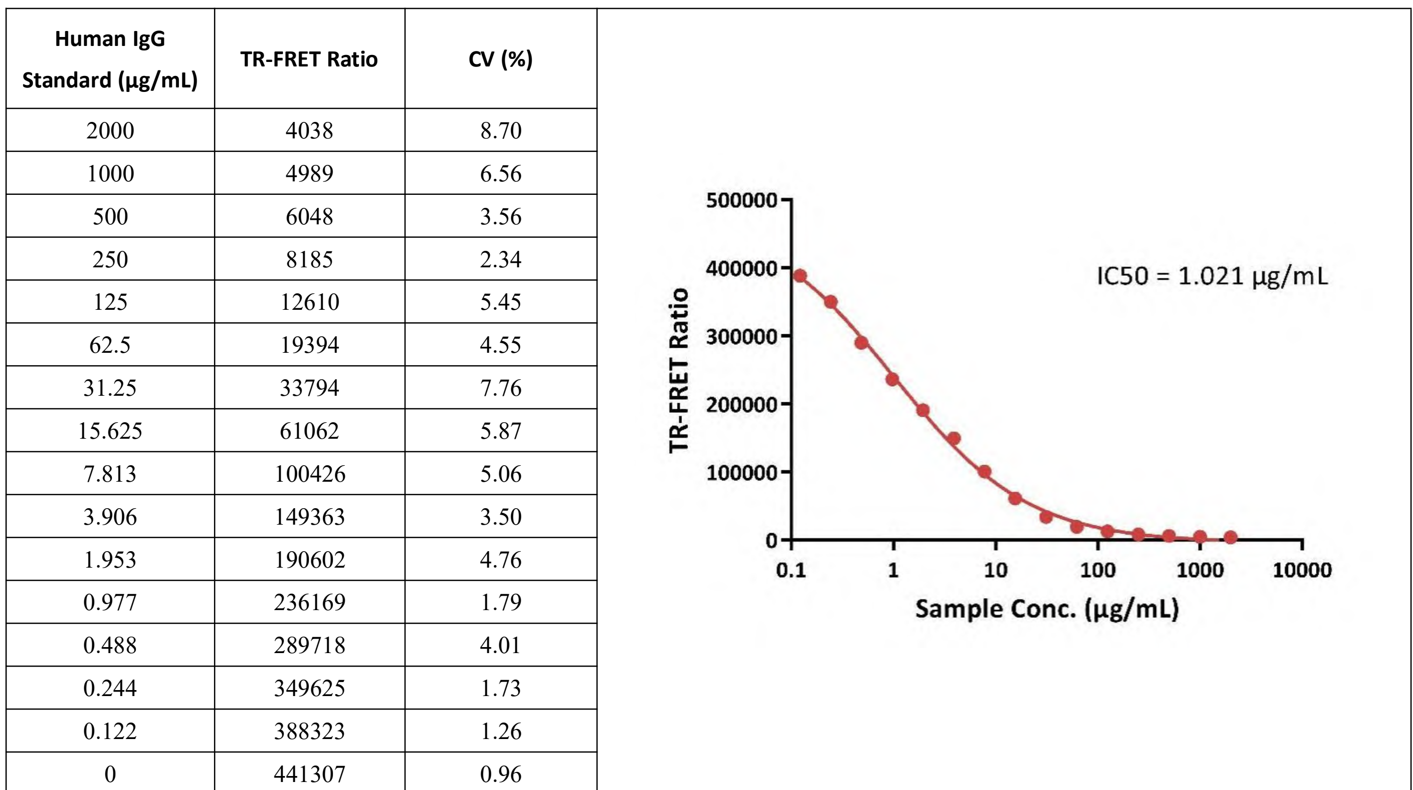
PRECAUTIONS

1. This kit is for research use only and is not intended for diagnostic or therapeutic applications.
2. Use this kit strictly according to the provided instructions.
3. Do not mix or substitute reagents with those from other kits or from kits of different lot numbers.
4. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals form in any buffer solution, incubate the solution in a 37℃-40℃ water bath for 15-20 minutes until the crystals are completely dissolved. Before use, bring the solution back to room temperature.
5. Store this kit according to the storage instructions provided. Do not use reagents past their expiration date.
6. Prepare the working solution of each component according to the experimental requirements. All prepared working solutions are **for single use only** and **must not be stored.**
7. Ensure the correct use of dilution buffers for each component. Do not dilute samples with the TR-FRET Detection Buffer, pH7.4 (DB-05). Do not dilute Donor or Acceptor reagents with the TR-FRET Sample Dilution Buffer, pH7.4

(DB-04).

TYPICAL DATA

For each experiment, a standard curve should be established for every microplate, and the specific Ratio values calculated may vary depending on the laboratories, operator, and equipment. Different microplate readers and different gain settings may produce varying fluorescence signals. Please adjust the parameters according to the user manual. Reduce the gain value if the signal is too high. The following data are obtained using the BMG LABTECH CLARIOstar® Plus and are provided for reference only.



Human IgG Standard (μg/mL)	TR-FRET Ratio	CV (%)
2000	4038	8.70
1000	4989	6.56
500	6048	3.56
250	8185	2.34
125	12610	5.45
62.5	19394	4.55
31.25	33794	7.76
15.625	61062	5.87
7.813	100426	5.06
3.906	149363	3.50
1.953	190602	4.76
0.977	236169	1.79
0.488	289718	4.01
0.244	349625	1.73
0.122	388323	1.26
0	441307	0.96

SENSITIVITY

The minimum detectable concentration was determined by subtracting two standard deviations from the mean Ratio value of twenty zero-standard replicates and calculating the corresponding concentration.

Assay range (μg/mL)	1.953 μg/mL-500 μg/mL
Limit of detection (LOD)	0.03 μg/mL
Limit of Quantitation (LOQ)	1.953 μg/mL

PRECISION AND ACCURACY

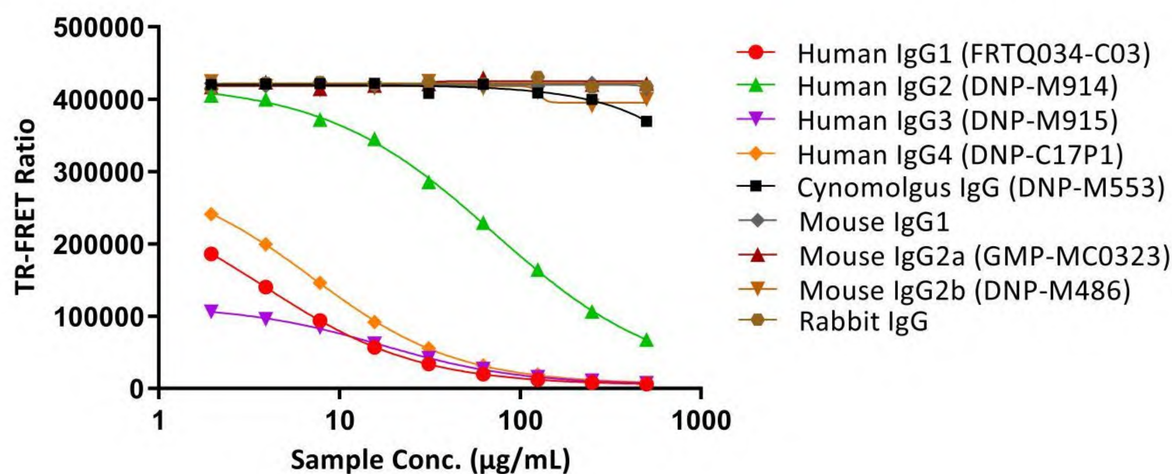
Intra-assay Precision and Accuracy: Five samples with known concentrations were tested repeatedly 16 times within a single analytical batch to assess within-batch precision and accuracy.

Inter-assay Precision and Accuracy: Five samples with known concentrations were tested repeatedly across three independent analytical batches to evaluate between-batch precision and accuracy.

	Intra-assay Precision and Accuracy					Inter-assay Precision and Accuracy				
Sample Conc. (μg/mL)	**500**	**375**	**50**	**5**	**1.953**	**500**	**375**	**50**	**5**	**1.953**
Number of Replicate	16	16	16	16	16	3	3	3	3	3
Mean (μg/mL)	472.98	371.13	53.83	4.82	1.92	463.02	358.00	50.17	4.60	1.86
Standard Deviation	69.76	43.14	3.40	0.31	0.14	67.43	31.46	4.25	0.37	0.13
CV (%)	14.75	11.63	6.31	6.48	7.26	14.56	8.79	8.47	8.00	7.06
Recovery (%)	94.60	98.97	107.66	96.49	98.11	92.60	95.47	100.34	91.95	95.36

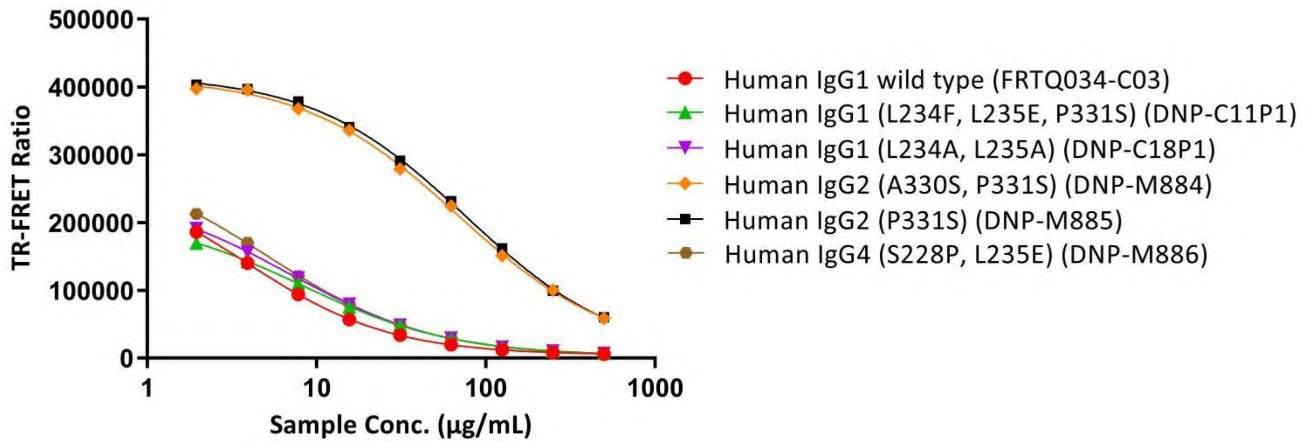
SPECIFICITY

The kit is human IgG specific and not compatible with cynomolgus IgG, mouse IgG1, mouse IgG2a, mouse IgG2b and rabbit IgG. Human IgG1, human IgG3 and IgG4 demonstrate excellent linearity within the range of 1.953-500 μg/mL, while human IgG2 is undetectable at concentrations approximately below 4 μg/mL.

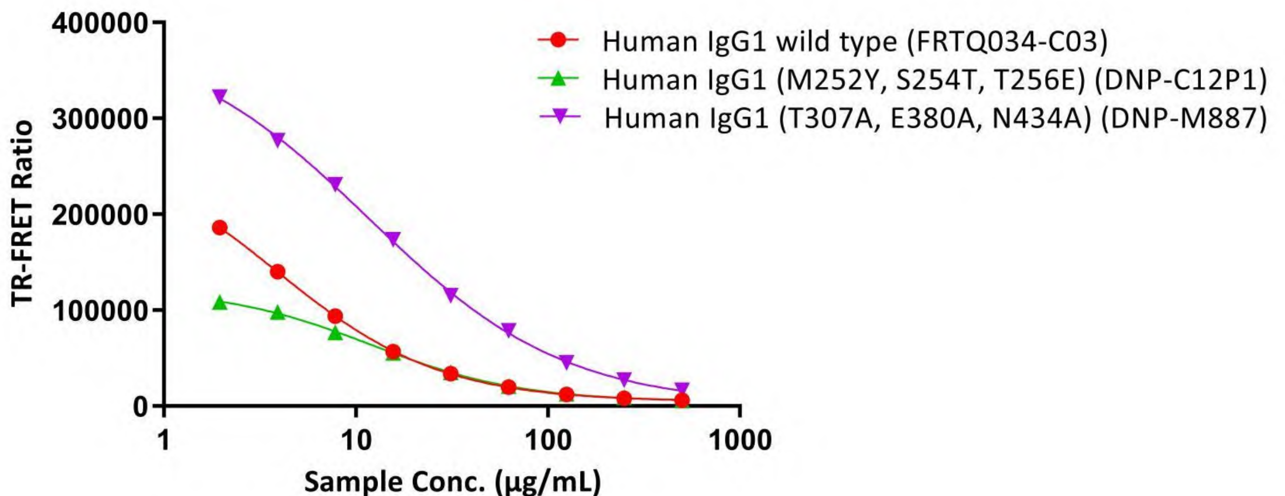


DETECTION OF FC-ENGINEERED ANTIBODIES

1. This kit can be used for the quantitative detection of Fc-engineered antibodies designed for **Elimination of Fc-mediated effector functions**. Human IgG1 and IgG4 demonstrate excellent linearity within the range of 1.953-500 μg/mL, while human IgG2 is undetectable at concentrations approximately below 4 μg/mL.



2. This kit can be used for the quantitative detection of Fc-engineered antibodies designed for **Half-life extension.** Detection results may vary depending on the specific mutation site. Please select the appropriate standard according to the mutation type of your sample.



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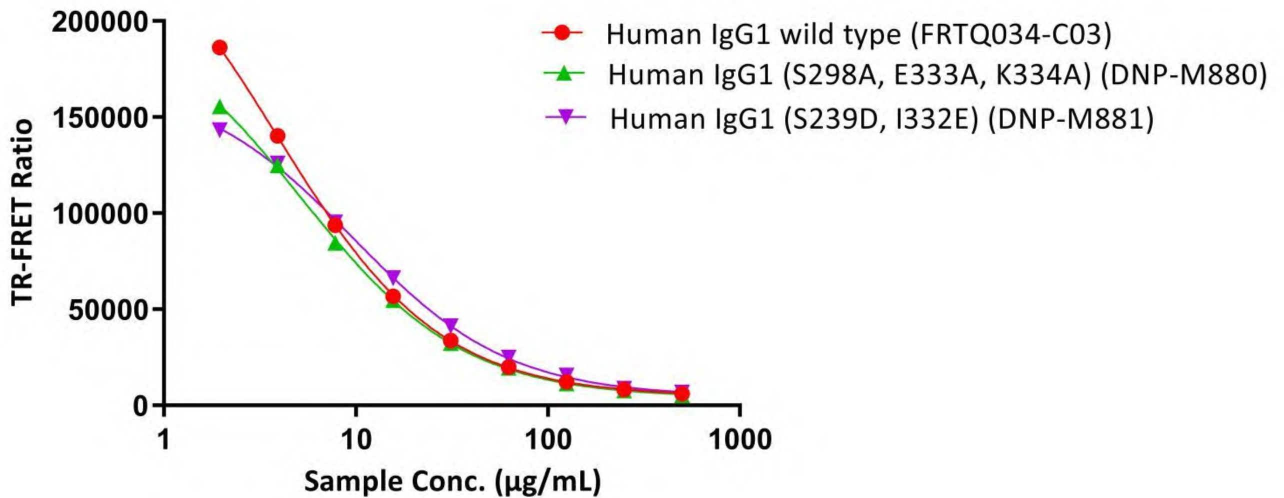
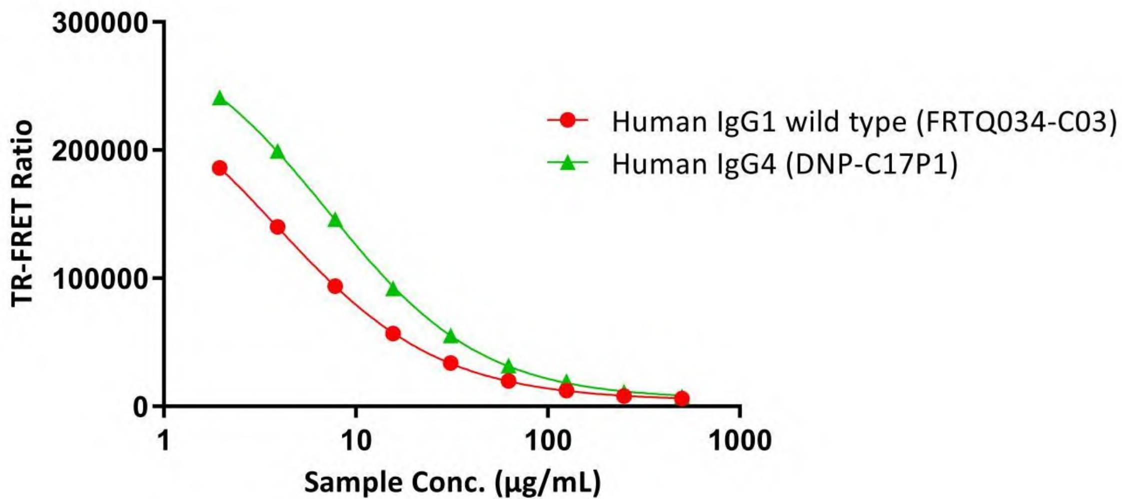
3. This kit can be used for the quantitative detection of Fc-engineered antibodies designed for the **Enhancement of ADCC activity**. Detection results may vary across different mutation sites. Please select the appropriate standard according to the specific mutation type of your sample.



4. This kit can be used for the quantitative detection of Fc-engineered antibodies designed for the **Hinge region stabilization**. Detection results may vary across different mutation sites. Please select the appropriate standard according to the specific mutation type of your sample.



LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IgG standard were serially diluted with TR-FRET Sample Dilution Buffer, pH 7.4 (DB-04), to generate samples with concentrations within the dynamic range of the assay.

Dilution factor		Cell culture medium		Empty vector-transfected cell supernatant	
		DMEM+10%FBS	RPMI1640+10%FBS	CHO	HEK293
1:4	Average Recovery (%)	78.23	87.82	85.98	85.26
	Range (%)	73.13-86.99	70.01-116.92	79.93-99.15	74.35-95.67
1:8	Average Recovery (%)	95.47	94.25	97.81	99.76
	Range (%)	85.50-104.39	86.33-99.74	92.59-105.84	83.19-109.09
1:16	Average Recovery (%)	94.05	98.35	105.20	106.45
	Range (%)	89.88-96.96	91.45-105.40	101.23-113.36	100.35-113.97
1:32	Average Recovery (%)	97.99	100.04	98.23	102.35
	Range (%)	95.10-101.15	97.07-103.90	90.38-103.51	98.28-108.56

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Large CV	* Inaccurate pipetting. * Air bubbles in wells.	* Check pipettes. * Remove bubbles in wells.
High background	* Reagent contamination. * Interfering components.	* Avoid contaminating the reagents. * Ensure the purity of the samples or dilute them to reduce interference.
Hook Effect	* Inappropriate sample detection concentration. * The usage concentration of the Donor/Acceptor is not applicable to certain special samples.	* Sample dilution optimization. * Optimize the usage concentration of Donor/Acceptor.
Very low readings across the plate	* Incorrect wavelengths or gain value set. * Insufficient reaction time.	* Check filters/gain/reader. * Increase reaction time.
Matrix interference	* Sample matrix does not match the standard curve.	* Dilute the sample using a higher dilution factor to reduce matrix interference, and perform spike recovery experiments to evaluate matrix effects. * Prepare the standard curve using the same matrix as the test sample to minimize matrix interference and obtain more accurate back-calculated sample concentrations.
Low instrument detection sensitivity	* Instrument parameters are not appropriately set.	* Set the instrument delay time to 50–100 μs and the integration time to 100–400 μs. * Adjust the instrument gain to an appropriate range to avoid signal oversaturation or excessively low signal levels.

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