Human IFN-gamma Detection Kit (TR-FRET)

Catalog Number：FRT-C01

Assay Tests: 100 tests & 500 tests

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

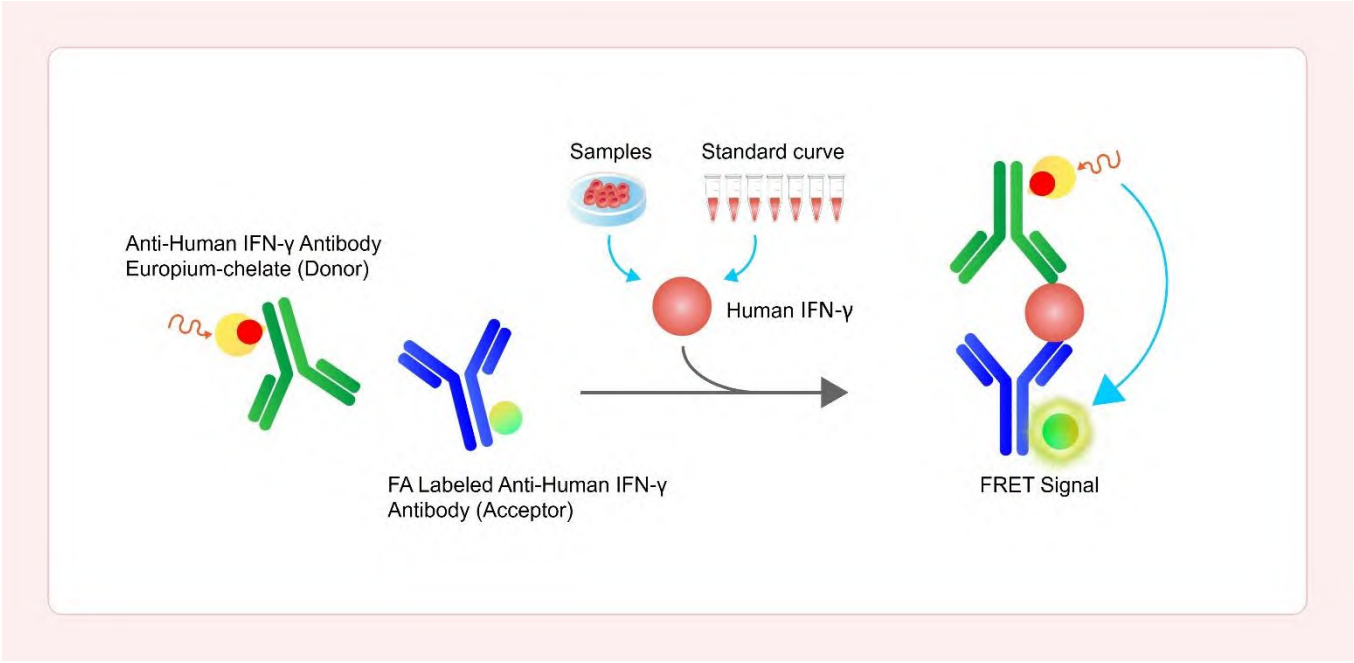
IMPORTANT: Please read this manual carefully before performing your experiment.

PRODUCT OVERVIEW

The Human IFN-gamma Detection Kit (TR-FRET) can detect the human IFN-gamma in homogeneous system within 2 hours, it is highly sensitive, short detection time, easy to use and possesses high-throughput screening capability. Compatible with microplate formats, this kit supports high-throughput screening workflows, enabling efficient large-scale sample analysis. This kit is specifically designed for the accurate quantitation of human IFN-gamma from cell culture supernatant, serum and plasma.

ASSAY PRINCIPLE

This Human IFN-gamma Detection Kit (TR-FRET) is based on a TR-FRET sandwich assay using two different specific antibodies. One antibody is labeled with a europium chelate (as the Donor), while the second is labeled with FA (as the Acceptor). In the presence of human IFN-gamma, the Donor and Acceptor are in close proximity because both labeled antibodies bind to the human IFN-gamma. Upon Donor excitation with light of a specific wavelength (337nm), in addition to Donor emission (620nm), non-radiative transfer of energy occurs between Donor and Acceptor, resulting in Acceptor emission (665nm).



NOTE:

1. For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures.
2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

CONTENTS AND STORAGE

Store the unopened kit at 2-8 °C. Do not use the kit after the expiration date indicated on the kit label.

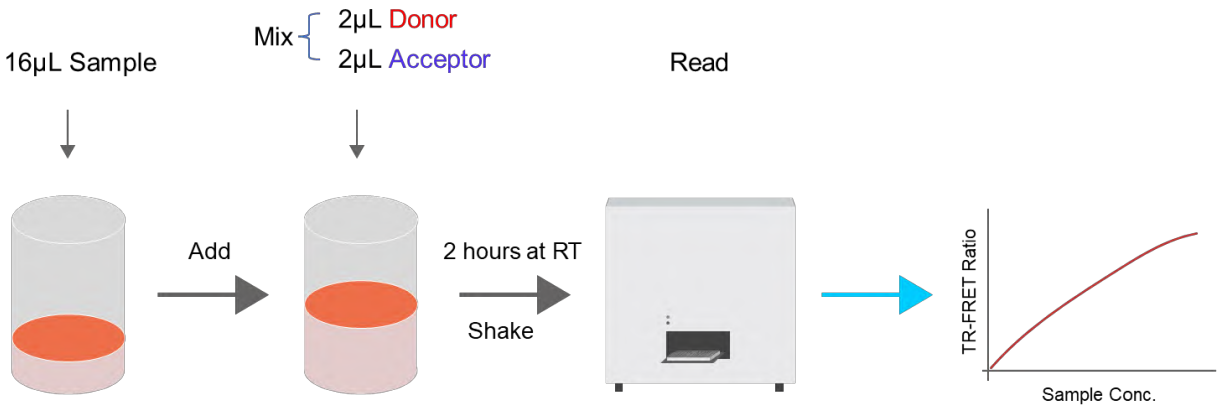
Catalog	Contents	Size (100 tests)	Size (500 tests)	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
FRTC01-C01	Anti-Human IFN-γ Antibody Europium-chelate	100 tests	500 tests	-70℃, protected from light
FRTC01-C02	FA Labeled Anti-Human IFN-γ Antibody	100 tests	500 tests	-70℃, protected from light
FRTC01-C03	Human IFN-γ Standard	20 μg	20 μg	-70℃
DB-04	TR-FRET Sample Dilution Buffer, pH7.4	50 mL	50 mL	2-8℃
DB-05	TR-FRET Detection Buffer, pH7.4	50 mL	50 mL	2-8℃

Note: Anti-Human IFN-γ Antibody Europium-chelate and FA Labeled Anti-Human IFN-γ Antibody should be protected from light.

REQUIRED MATERIALS NOT SUPPLIED

	Items	Specifications
Instrument	Microplate reader	Plate reader capable of measuring signals at 665nm/620nm in TR-FRET mode. Example: BMG LABTECH CLARIOstar® Plus.
	Microplate shaker	-
Reagents	Deionized, ultrapure or distilled water	-
Consumables	Single channel and multi-channel pipettes	There must be calibrated pipettes, with 10 μL, 200 μL and 1000 μL 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 give the lowest background signal. e.g., 384-well white plate (iSTAR, Cat. No. GT247.008).
	EP Tubes	For to prepare standard dilutions and working solutions.

QUICK GUIDE



REAGENT PREPARATION

1. Bring all reagents up to room temperature (20℃-25℃) before use. If crystals have formed in the buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.
2. Reconstitute provided lyophilized materials with **deionized, ultrapure or distilled water** as described in table 1 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.
3. Avoid vigorous shaking or vortexing. The reconstituted stock solutions should be stored at -70℃. It is recommended not to freeze-thaw more than twice.

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

Table 1. Reconstitution methods for 100 tests and 500tests

Catalog	Components	The volume of buffer required for reconstitution		Stock Solution Conc.
		Size (100 tests)	Size (500 tests)	
FRTC01-C01	Anti-Human IFN-γ Antibody Europium-chelate	60 μL water	300 μL water	/
FRTC01-C02	FA Labeled Anti-Human IFN-γ Antibody	60 μL water	300 μL water	/
FRTC01-C03	Human IFN-γ Standard	100 μL water	100 μL water	200 μg/mL

PROCEDURE OF ASSAY

- Each well requires 16 µL of standard or sample, and 4 µL of the pre-mix Donor & Acceptor solution.
- The stock standard solution was serially diluted with **either DB-04 (TR-FRET Sample Dilution Buffer, pH 7.4) or the blank matrix identical to that of the test samples**.
- It is recommended to prepare standard curves and perform sample dilution using the blank matrix consistent with that of the test samples, so as to minimize interference from potential **matrix effects** and obtain more accurate calculated sample concentrations.
- If the blank matrix identical to that of the test samples is unavailable, DB-04 (TR-FRET Sample Dilution Buffer, pH 7.4) may be used as the surrogate matrix for standard curve preparation and sample dilution. In this case, the **parallelism** between the surrogate matrix and the matrix of the test samples, as well as the **minimum required dilution (MRD)**, shall be verified.

1. Prepare standard serial dilutions.
   - Label a tube "**Stock 1**". Add 2 µL of the reconstituted human IFN-gamma Standard (200 µg/mL) and 398 µL of **Sample Dilution Buffer (DB-04 or a solution with the same matrix as the samples)** to tube **Stock 1**, gently mix well.
   - Label a tube "**Stock 2**". Add 40 µL of **Stock 1** and 360 µL of **Sample Dilution Buffer** to tube **Stock 2**, gently mix well.
   - Label 7 tubes, one for each standard point: Std 1, Std 2, Std 3, Std 4, Std 5, Std 6, Std 7.
   - Add 20 µL of the liquid from **Stock 2** and 480 µL of **Sample Dilution Buffer** to tube Std 7, thoroughly mix (Std 7 = 4000 pg/mL).
   - Prepare the standard curve as follows: Add 220 µL of **Sample Dilution Buffer** to each tube (Std 1, Std 2, Std 3, Std 4, Std 5, Std 6).
   - Transfer 200 µL of liquid from Std 7 to the tube Std 6, and thoroughly mix (Std 6 = 1904.8 pg/mL).
   - Continue to transfer 200 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std 1.
   - Sample Dilution Buffer serves as zero standard (blank).

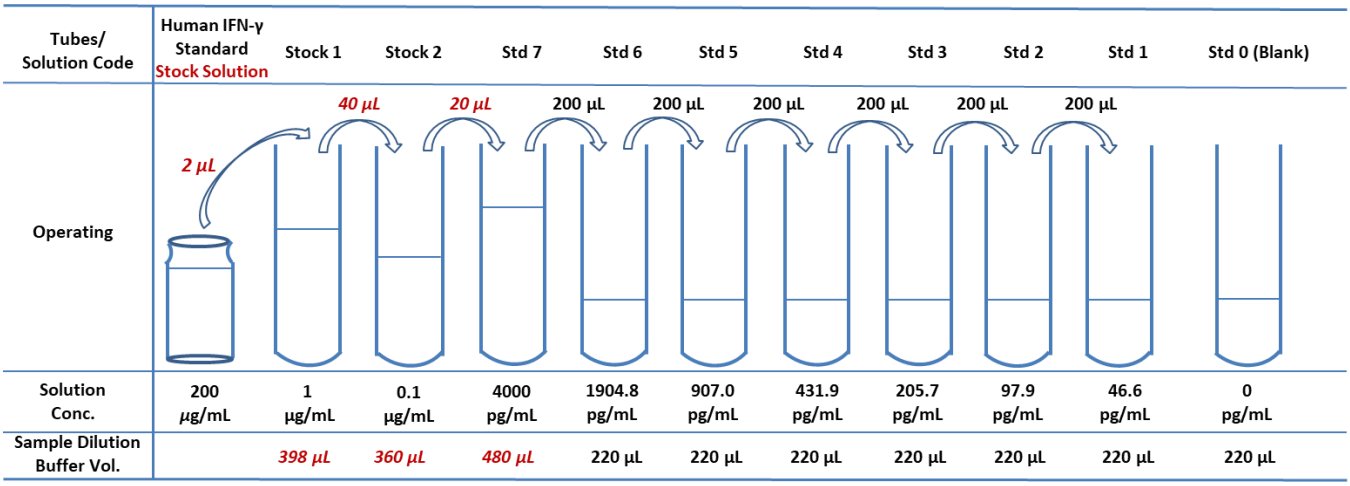


Figure 1. Preparation of serial dilutions of the human IFN-gamma standard

2. Prepare Biological Samples. Each well requires **16 µL** of sample. All samples with a concentration above the highest standard (Std 7) must be diluted in **Sample Dilution Buffer**.
3. Prepare Donor Working Solution. Dilute the Anti-Human IFN-γ Antibody Europium-chelate (FRTC01-C01) stock solution 4-fold with TR-FRET Detection Buffer, pH7.4 (DB-05).
4. Prepare Acceptor Woking Solution. Dilute the FA Labeled Anti-Human IFN-γ Antibody (FRTC01-C02) stock solution 4-fold with TR-FRET Detection Buffer, pH7.4 (DB-05).
5. Prepare the pre-mix solution. Pre-mix the Donor and Acceptor working solution 1:1 (v/v).
6. Add 16 µL of standards or samples, 4 µL of the pre-mix Donor & Acceptor solution to each well. Refer to **followed figure 2 and Table 2** for the design of microplate layout according to the experimental requirements and add the corresponding reaction solution into the corresponding plate holes
7. Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for **2 hours** on orbital shaker at 400-600 rpm.
8. Data Recording. Use the TR-FRET module of a microplate reader to read the fluorescence signal at 665nm and 620nm (the excitation wavelength is 337 nm).

Note:

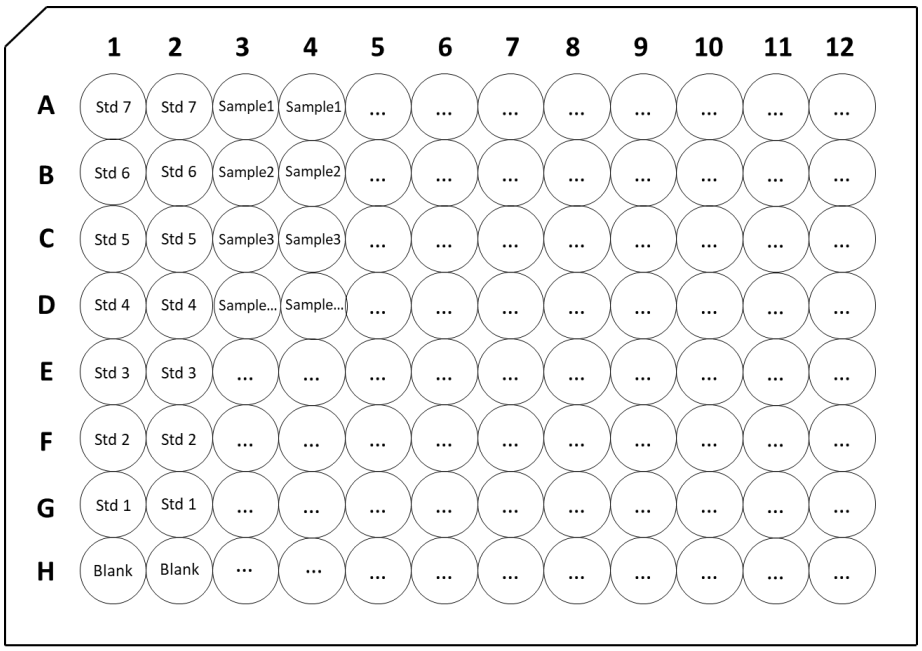


Figure 2. Plate layout

Table 2. Samples adding to microplate

	1	2	3	4
A	16 μL Std 7 4 μL pre-mixed IFN-γ antibodies	16 μL Std 7 4 μL pre-mixed IFN-γ antibodies	16 μL Sample1 4 μL pre-mixed IFN-γ antibodies	16 μL Sample1 4 μL pre-mixed IFN-γ antibodies
B	16 μL Std 6 4 μL pre-mixed IFN-γ antibodies	16 μL Std 6 4 μL pre-mixed IFN-γ antibodies	16 μL Sample2 4 μL pre-mixed IFN-γ antibodies	16 μL Sample2 4 μL pre-mixed IFN-γ antibodies
C	16 μL Std 5 4 μL pre-mixed IFN-γ antibodies	16 μL Std 5 4 μL pre-mixed IFN-γ antibodies	16 μL Sample3 4 μL pre-mixed IFN-γ antibodies	16 μL Sample3 4 μL pre-mixed IFN-γ antibodies
D	16 μL Std 4 4 μL pre-mixed IFN-γ antibodies	16 μL Std 4 4 μL pre-mixed IFN-γ antibodies	16 μL Sample... 4 μL pre-mixed IFN-γ antibodies	16 μL Sample... 4 μL pre-mixed IFN-γ antibodies
E	16 μL Std 3 4 μL pre-mixed IFN-γ antibodies	16 μL Std 3 4 μL pre-mixed IFN-γ antibodies	...	...
F	16 μL Std 2 4 μL pre-mixed IFN-γ antibodies	16 μL Std 2 4 μL pre-mixed IFN-γ antibodies	...	...
G	16 μL Std 1 4 μL pre-mixed IFN-γ antibodies	16 μL Std 1 4 μL pre-mixed IFN-γ antibodies	...	...
H	16 μL Sample Dilution Buffer 4 μL pre-mixed IFN-γ antibodies	16 μL Sample Dilution Buffer 4 μL pre-mixed IFN-γ antibodies	...	...

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 and subtract average zero standard Ratio to obtain the ΔRatio.

3. The standard curve is plotted with the standard concentration as x-axis and the ΔRatio 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: $R^2 \geqslant 0.9900$.

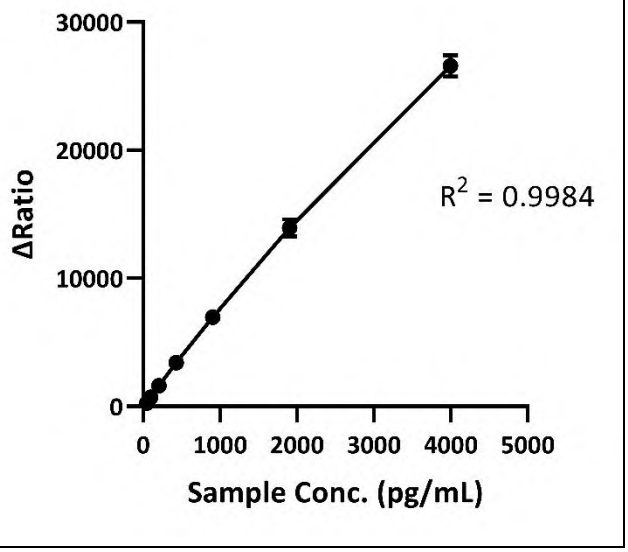
4. Detection range: 46.6 pg/mL-4000 pg/mL. If the ΔRatio of a test sample is higher than the ΔRatio corresponding to the 4000 pg/mL standard, the analyte concentration of the sample is above the upper limit of quantification. The sample should be diluted with the dilution buffer and re-assayed. If the ΔRatio of a test sample is lower than ΔRatio corresponding to the 46.6 pg/mL standard, the analyte concentration is below the lower limit of quantification and should be reported as < 46.6 pg/mL.

TYPICAL DATA

Note: 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. *The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

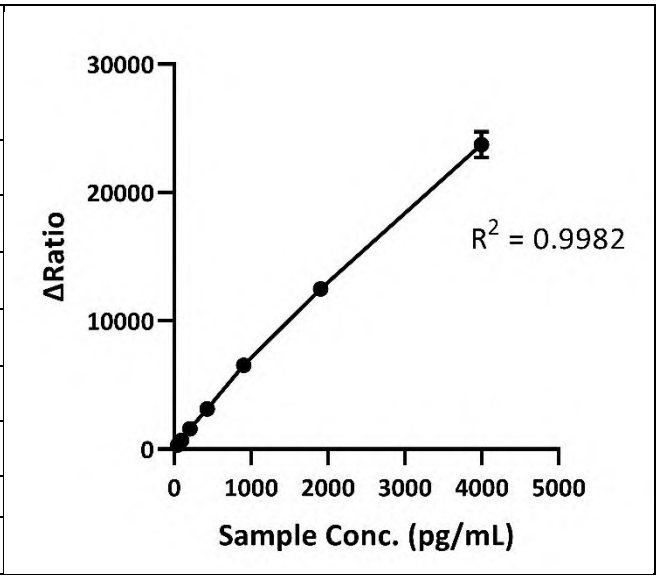
Standard curve generated in TR-FRET Sample Dilution Buffer, pH7.4 (DB-04):

Human IFN-gamma Standard (pg/mL)	TR-FRET Ratio	Δ Ratio	CV (%)
4000	27777	26583	2.95
1904.8	15122	13928	4.48
907.0	8152	6958	3.09
431.9	4613	3419	7.05
205.7	2819	1625	0.81
97.9	1912	718	6.48
46.6	1459	265	2.44
0	1194	0	3.67



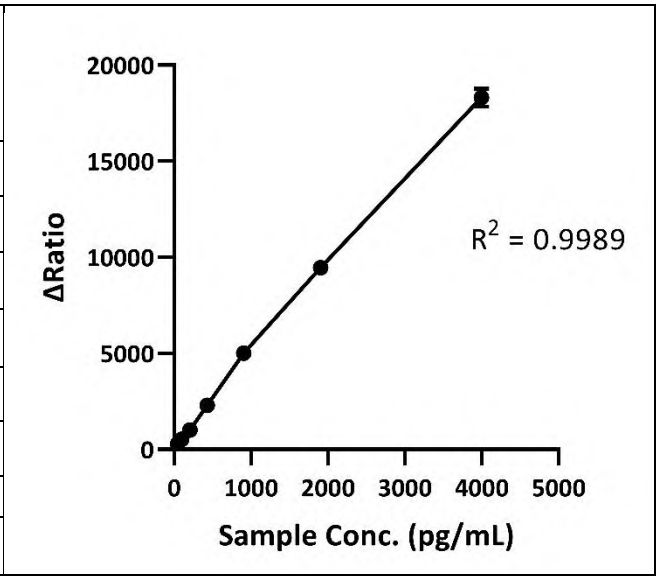
Standard curve generated in DMEM with 10% FBS:

Human IFN-gamma Standard (pg/mL)	TR-FRET Ratio	Δ Ratio	CV (%)
4000	25496	23803	3.95
1904.8	14253	12560	1.85
907.0	8297	6604	1.44
431.9	4894	3201	1.73
205.7	3362	1669	2.29
97.9	2454	761	4.26
46.6	2112	420	2.05
0	1693	0	4.17



Standard curve generated in RPMI 1640 with 10% FBS:

Human IFN-gamma Standard (pg/mL)	TR-FRET Ratio	Δ Ratio	CV (%)
4000	19782	18308	2.32
1904.8	10937	9463	0.46
907.0	6474	5000	2.96
431.9	3769	2295	6.05
205.7	2485	1011	1.75
97.9	1997	523	2.01
46.6	1773	299	7.61
0	1474	0	1.81



PERFORMANCE CHARACTERISTICS

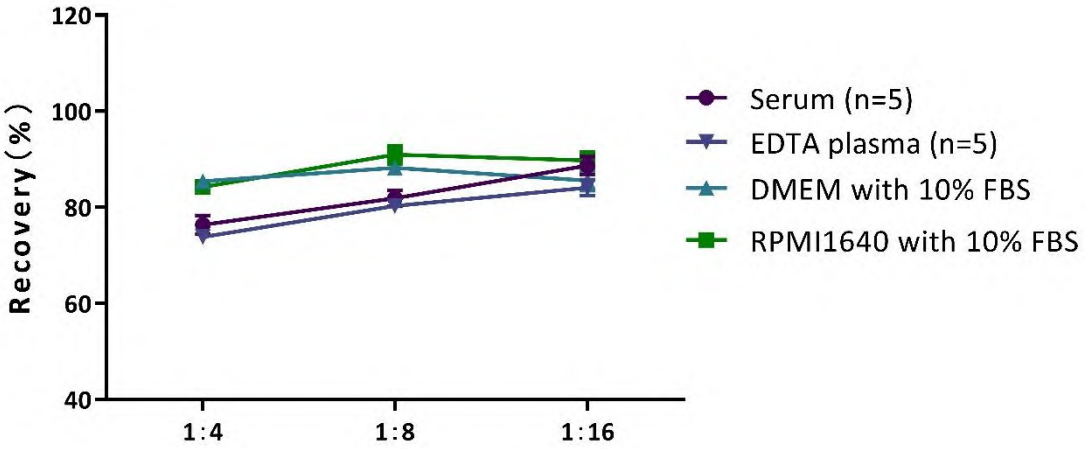
Sensitivity

The minimum detectable concentration was determined by adding twice standard deviations to the Ratio of twenty zero standard replicates and calculating the corresponding concentration.

Matrix	TR-FRET Sample Dilution Buffer, pH7.4 (DB-04)	DMEM with 10% FBS	RPMI 1640 with 10% FBS
Assay range	46.6 pg/mL-4000 pg/mL	46.6 pg/mL-4000 pg/mL	46.6 pg/mL-4000 pg/mL
Limit of detection (LOD)	18.70 pg/mL	25.47 pg/mL	33.73 pg/mL
Limit of Quantitation (LOQ)	46.6pg/mL	46.6 pg/mL	46.6 pg/mL

Linearity

To evaluate the **linearity** of the assay, samples spiked with high-concentration human IFN-gamma in various matrices were prepared. With DB-04 (TR-FRET Sample Dilution Buffer, pH 7.4) as the **surrogate matrix**, standard curves were generated and the spiked samples were subjected to serial gradient dilution, followed by the quantification of the spiked samples.



Inter-Assay/Intra-Assay 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. (pg/mL)	**4000**	**3000**	**750**	**97.9**	**46.6**	**4000**	**3000**	**750**	**97.9**	**46.6**
Number of Replicate	16	16	16	16	16	3	3	3	3	3
Mean (pg/mL)	3962	2985	802	96	44	3963	2998	800	100	45
Standard Deviation	121.37	87.19	29.25	12.52	12.04	4.98	20.79	16.21	4.39	8.20
CV (%)	3.06	2.92	3.65	13.10	27.54	0.13	0.69	2.03	4.37	18.08
Recovery (%)	99.05	99.48	106.93	97.62	93.72	99.08	99.93	106.67	102.57	97.30

Calibration

This immunoassay is calibrated against a highly purified HEK293 cell-expressed recombinant human IFN-gamma produced at ACROBiosystems. To convert sample values obtained with the Human IFN-gamma Detection Kit (TR-FRET) to approximate NIBSC (82/587) nominally assigned mass values, use the equation below.

NIBSC/WHO (82/587) approximate value (IU/mL) = 0.03 × ACRO Human IFN-gamma value (pg/mL).

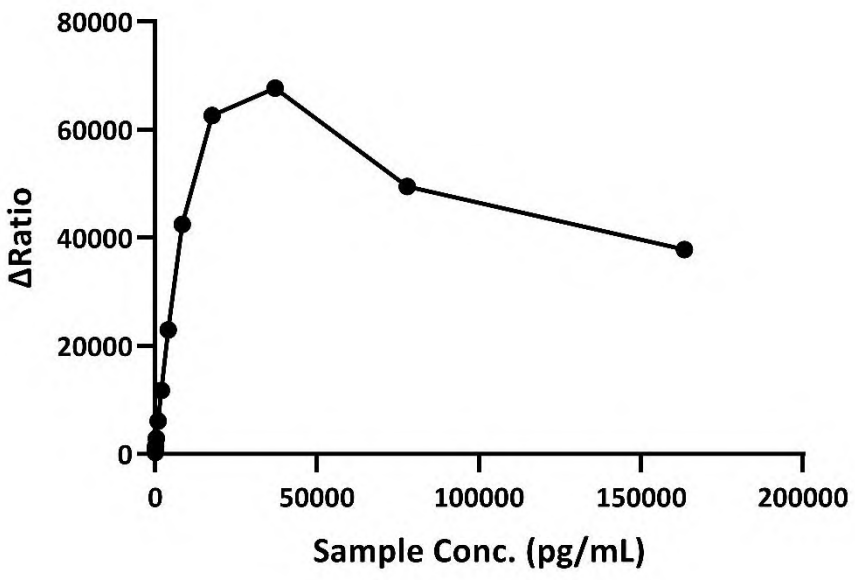
Specificity

No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines at up to 1 μg/mL.

Human	Mouse
IL-10	IL-10
IL-6	IL-6
TNF-alpha	TNF-alpha
IL-2	IL-2
	IFN-gamma

Hook Effect

Not be affected by the concentration of human IFN-gamma up to 37044 pg/mL.



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.