



Human IL-2 Detection Kit (TR-FRET)

Catalog Number: FRT-C03

Assay Tests: 100 tests & 500 tests

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

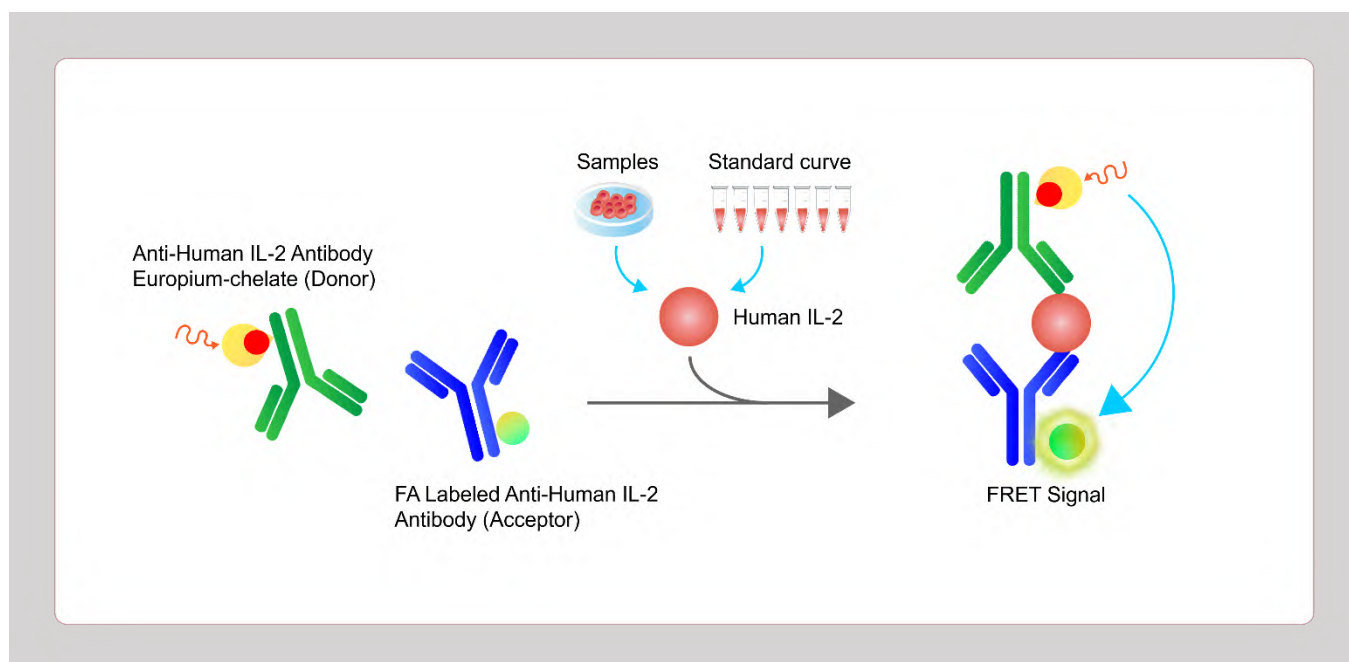
IMPORTANT: Please read this manual carefully before performing your experiment.

PRODUCT OVERVIEW

Human IL-2 Detection Kit (TR-FRET) can detect the human IL-2 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 IL-2 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 IL-2, the Donor and Acceptor are in close proximity because both labeled antibodies bind to the human IL-2. 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.

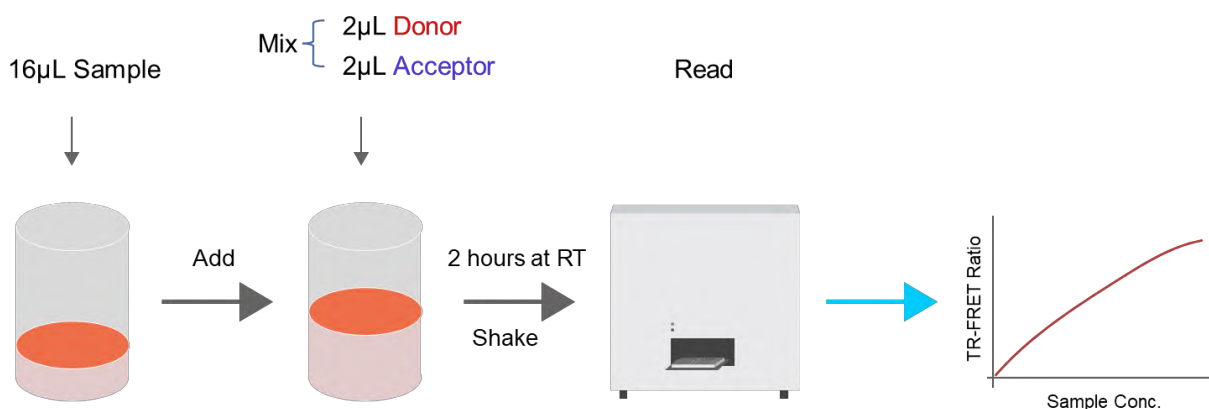
Catalog	Contents	Size (100 tests)	Size (500 tests)	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
FRTC03-C01	Anti-Human IL-2 Antibody Europium-chelate	100 tests	500 tests	-70°C, protected from light
FRTC03-C02	FA Labeled Anti-Human IL-2 Antibody	100 tests	500 tests	-70°C, protected from light
FRTC03-C03	Human IL-2 Standard	15 µg	15 µg	-70°C
DB-04	TR-FRET Sample Dilution Buffer, pH7.4	50 mL	50 mL	2-8°C
DB-05	TR-FRET Detection Buffer, pH7.4	50 mL	50 mL	2-8°C

Note: Anti-Human IL-2 Antibody Europium-chelate and FA Labeled Anti-Human IL-2 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°C-25°C) 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°C. It is recommended not to freeze-thaw more than twice.

Note: Both the Anti-Human IL-2 Antibody Europium-chelate and FA Labeled Anti-Human IL-2 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)	
FRTC03-C01	Anti-Human IL-2 Antibody Europium-chelate	60 µL water	300 µL water	/
FRTC03-C02	FA Labeled Anti-Human IL-2 Antibody	60 µL water	300 µL water	/
FRTC03-C03	Human IL-2 Standard	150 µL water	150 µL water	100 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 IL-2 Standard (100 $\mu\text{g}/\text{mL}$) and 198 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 8 tubes, one for each standard point: Std 1, Std 2, Std 3, Std 4, Std 5, Std 6, Std 7, Std 8.
- Add 2 μL of the liquid from **Stock 1** and 248 μL of **Sample Dilution Buffer** to tube Std 8, thoroughly mix (Std 8 = 8000 pg/mL).
- Prepare the standard curve as follows: Add 100 μL of **Sample Dilution Buffer** to each tube (Std 1, Std 2, Std 3, Std 4, Std 5, Std 6, Std 7).
- Transfer 100 μL of liquid from Std 8 to the tube Std 7, and thoroughly mix (Std 7 = 4000 pg/mL).
- Continue to transfer 100 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).

Tubes/ Solution Code	Human IL-2 Standard Stock Solution	Stock 1	Std 8	Std 7	Std 6	Std 5	Std 4	Std 3	Std 2	Std 1	Std 0 (Blank)
Operating											
Solution Conc.	100 µg/mL	1 µg/mL	8000 pg/mL	4000 pg/mL	2000 pg/mL	1000 pg/mL	500 pg/mL	250 pg/mL	125 pg/mL	62.5 pg/mL	0 pg/mL
Sample Dilution Buffer Vol.		198 µL	248 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL

Figure 1. Preparation of serial dilutions of the human IL-2 standard

- Prepare Biological Samples. Each well requires **16 µL** of sample. All samples with a concentration above the highest standard (Std 8) must be diluted in **Sample Dilution Buffer**.
- Prepare Donor Working Solution. Dilute the Anti-Human IL-2 Antibody Europium-chelate (FRTC03-C01) stock solution 4-fold with TR-FRET Detection Buffer, pH7.4 (DB-05).
- Prepare Acceptor Working Solution. Dilute the FA Labeled Anti-Human IL-2 Antibody (FRTC03-C02) stock solution 4-fold with TR-FRET Detection Buffer, pH7.4 (DB-05).
- Prepare the pre-mix solution. Pre-mix the Donor and Acceptor working solution 1:1 (v/v).
- 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
- Seal the plate with microplate sealing film and incubate at room temperature (20°C-25°C) for **2 hours** on orbital shaker at 400-600 rpm.
- 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:

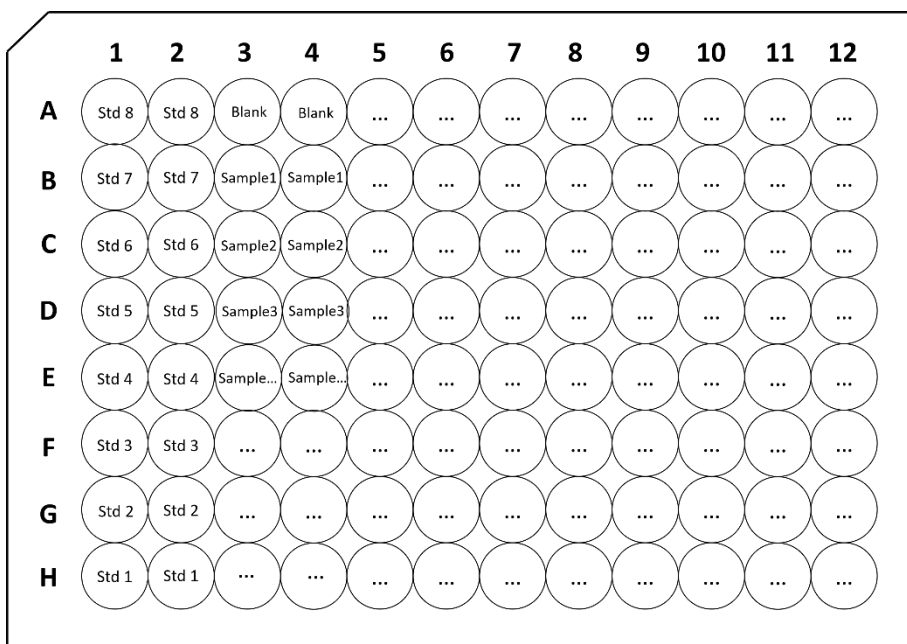


Figure 2. Plate layout

Table 2. Samples adding to microplate

	1	2	3	4
A	16 µL Std 8 4 µL pre-mixed IL-2 antibodies	16 µL Std 8 4 µL pre-mixed IL-2 antibodies	16 µL Sample Dilution Buffer 4 µL pre-mixed IL-2 antibodies	16 µL Sample Dilution Buffer 4 µL pre-mixed IL-2 antibodies
B	16 µL Std 7 4 µL pre-mixed IL-2 antibodies	16 µL Std 7 4 µL pre-mixed IL-2 antibodies	16 µL Sample1 4 µL pre-mixed IL-2 antibodies	16 µL Sample1 4 µL pre-mixed IL-2 antibodies
C	16 µL Std 6 4 µL pre-mixed IL-2 antibodies	16 µL Std 6 4 µL pre-mixed IL-2 antibodies	16 µL Sample2 4 µL pre-mixed IL-2 antibodies	16 µL Sample2 4 µL pre-mixed IL-2 antibodies
D	16 µL Std 5 4 µL pre-mixed IL-2 antibodies	16 µL Std 5 4 µL pre-mixed IL-2 antibodies	16 µL Sample3 4 µL pre-mixed IL-2 antibodies	16 µL Sample3 4 µL pre-mixed IL-2 antibodies
E	16 µL Std 4 4 µL pre-mixed IL-2 antibodies	16 µL Std 4 4 µL pre-mixed IL-2 antibodies	16 µL Sample... 4 µL pre-mixed IL-2 antibodies	16 µL Sample... 4 µL pre-mixed IL-2 antibodies
F	16 µL Std 3 4 µL pre-mixed IL-2 antibodies	16 µL Std 3 4 µL pre-mixed IL-2 antibodies
G	16 µL Std 2 4 µL pre-mixed IL-2 antibodies	16 µL Std 2 4 µL pre-mixed IL-2 antibodies
H	16 µL Std 1 4 µL pre-mixed IL-2 antibodies	16 µL Std 1 4 µL pre-mixed IL-2 antibodies

CALCULATION OF RESULTS

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

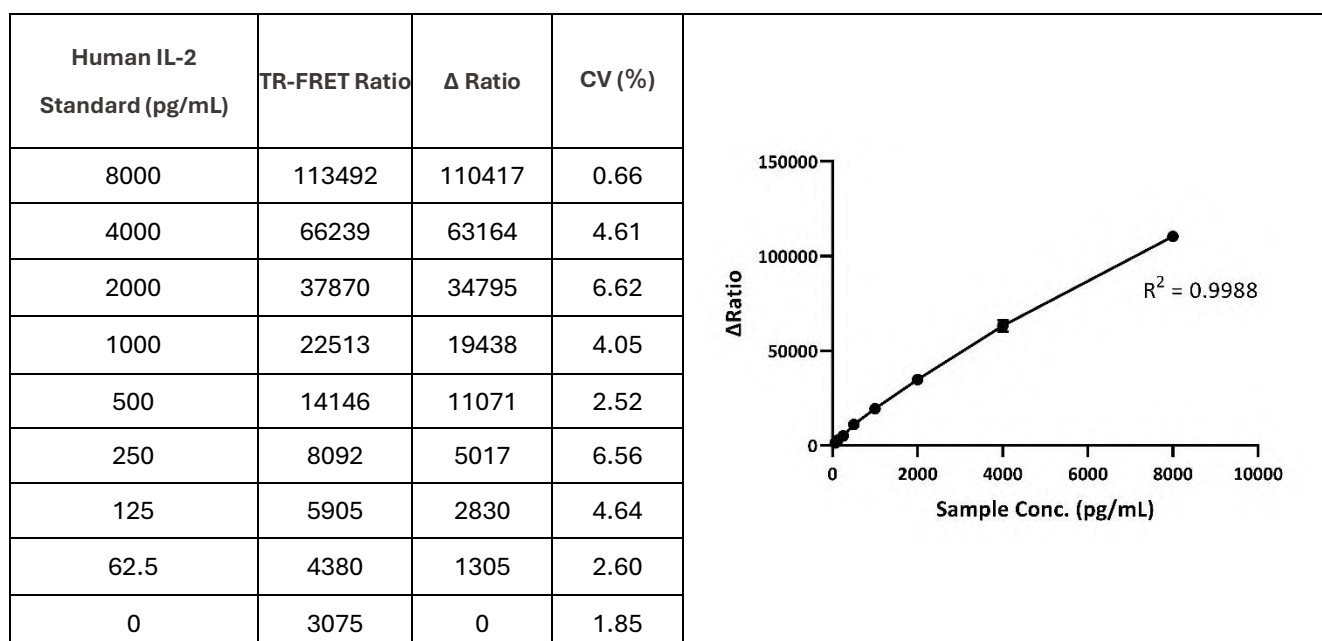
$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4.$$

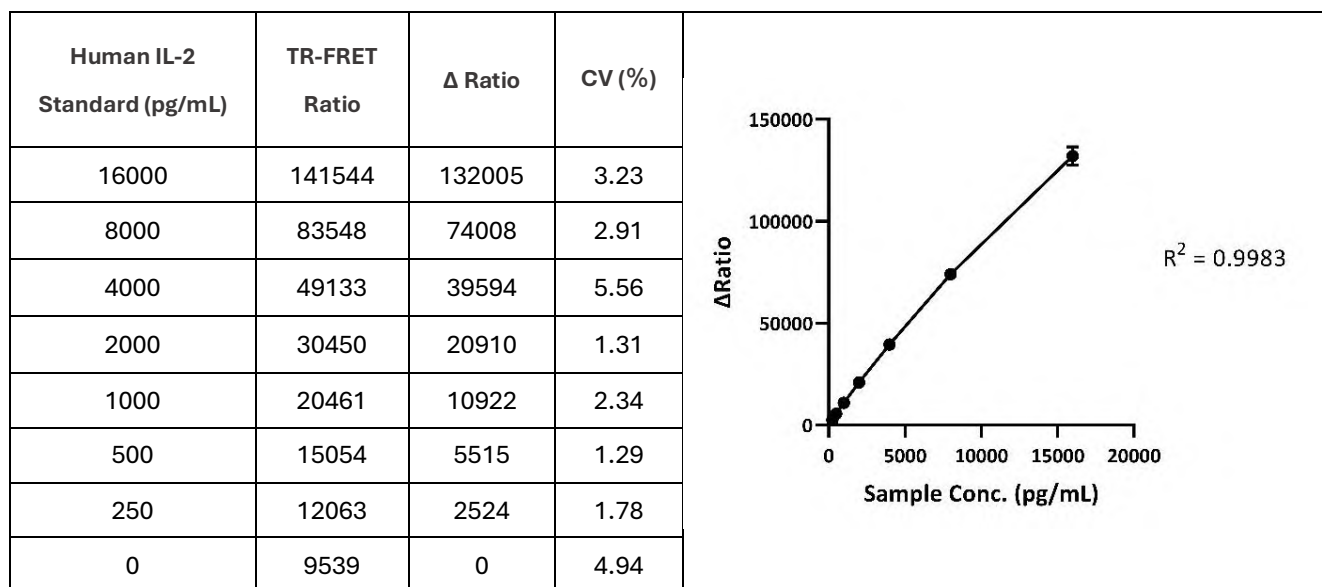
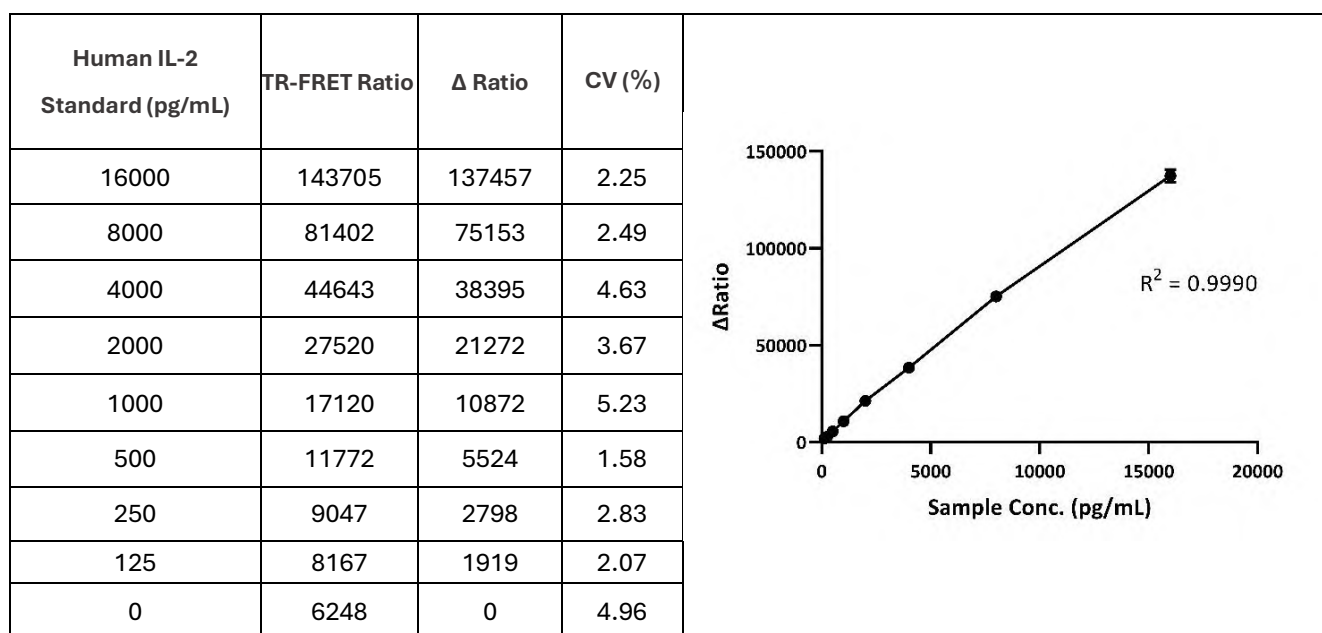
- Calculate the Mean Ratio for each sample, standard or control and subtract average zero standard Ratio to obtain the Δ Ratio.
- The standard curve is plotted with the standard concentration as x-axis and the Δ Ratio as y-axis. Establish a standard curve by processing the data using computer software capable of executing a **four-parameter logistic (4-PL)** curve fitting. Normal range of Standard curve: $R^2 \geq 0.9900$.
- Detection range: 62.5 pg/mL-8000 pg/mL. If the Δ Ratio of a test sample is higher than the Δ Ratio corresponding to the 8000 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 Δ Ratio of a test sample is lower than Δ Ratio corresponding to the 62.5 pg/mL standard, the analyte concentration is below the lower limit of quantification and should be reported as < 62.5 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):



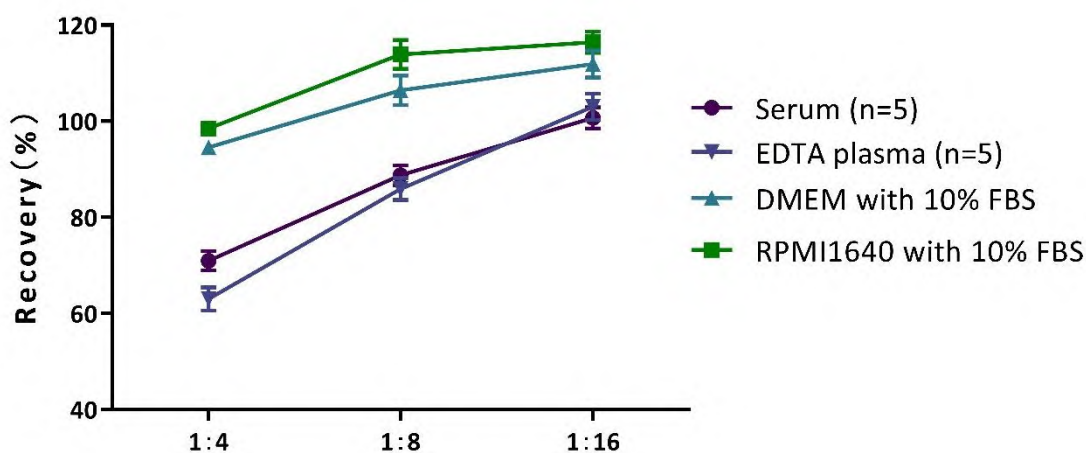
Standard curve generated in DMEM with 10% FBS:**Standard curve generated in RPMI 1640 with 10% FBS:****PERFORMANCE CHARACTERISTICS****Sensitivity**

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	62.5 pg/mL-8000 pg/mL	125 pg/mL-16000 pg/mL	125 pg/mL-16000 pg/mL
Limit of detection (LOD)	24.00 pg/mL	120.01 pg/mL	122.86 pg/mL
Limit of Quantitation (LOQ)	62.5 pg/mL	250 pg/mL	125 pg/mL

Linearity

To evaluate the **linearity** of the assay, samples spiked with high-concentration human IL-2 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				
	8000	6000	1500	125	62.5	8000	6000	1500	125	62.5
Sample Conc. (pg/mL)	8000	6000	1500	125	62.5	8000	6000	1500	125	62.5
Number of Replicate	16	16	16	16	16	3	3	3	3	3
Mean (pg/mL)	6451	5054	1570	149	53	7127	5483	1521	130	52
Standard Deviation	125.18	152.60	73.67	21.24	12.31	753.00	473.49	42.14	16.03	0.40
CV (%)	1.94	3.02	4.69	14.28	23.43	10.57	8.64	2.77	12.29	0.76
Recovery (%)	80.64	84.23	104.64	118.97	84.08	89.09	91.39	101.40	104.35	83.84

Calibration

This immunoassay is calibrated against a highly purified HEK293 cell-expressed recombinant human IL-2 produced at ACROBiosystems. To convert sample values obtained with the Human IL-2 Detection kit (TR-FRET) to approximate NIBSC (86/500) nominally assigned mass values, use the equation below.

$$\text{NIBSC/WHO (86/500) approximate value (IU/mL)} = 0.03 \times \text{ACRO Human IL-2 value (pg/mL)}.$$

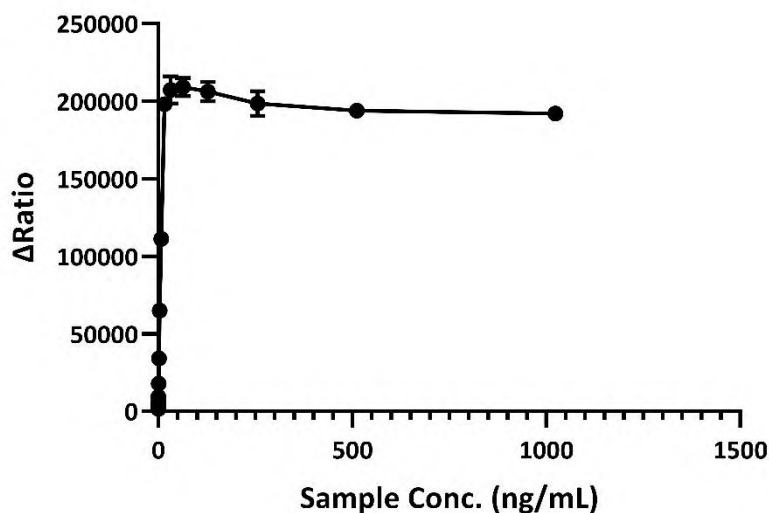
Specificity

No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines at up to 1 $\mu\text{g/mL}$.

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

Hook Effect

Not be affected by the concentration of human IL-2 up to 1024 ng/mL.



TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Large CV	<ul style="list-style-type: none"> * Inaccurate pipetting. * Air bubbles in wells. 	<ul style="list-style-type: none"> * Check pipettes. * Remove bubbles in wells.
High background	<ul style="list-style-type: none"> * Reagent contamination. * Interfering components. 	<ul style="list-style-type: none"> * Avoid contaminating the reagents. * Ensure the purity of the samples or dilute them to reduce interference.
Hook Effect	<ul style="list-style-type: none"> * Inappropriate sample detection concentration. * The usage concentration of the Donor/Acceptor is not applicable to certain special samples. 	<ul style="list-style-type: none"> * Sample dilution optimization. * Optimize the usage concentration of Donor/Acceptor.
Very low readings across the plate	<ul style="list-style-type: none"> * Incorrect wavelengths or gain value set. * Insufficient reaction time. 	<ul style="list-style-type: none"> * Check filters/gain/reader. * Increase reaction time.
Matrix interference	<ul style="list-style-type: none"> * Sample matrix does not match the standard curve. 	<ul style="list-style-type: none"> * 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	<ul style="list-style-type: none"> * Instrument parameters are not appropriately set. 	<ul style="list-style-type: none"> * 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.