

Mouse TNF-alpha Detection Kit (TR-FRET)

Pack Size: 100 tests & 500 tests

Catalog Number: FRT-B102

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

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

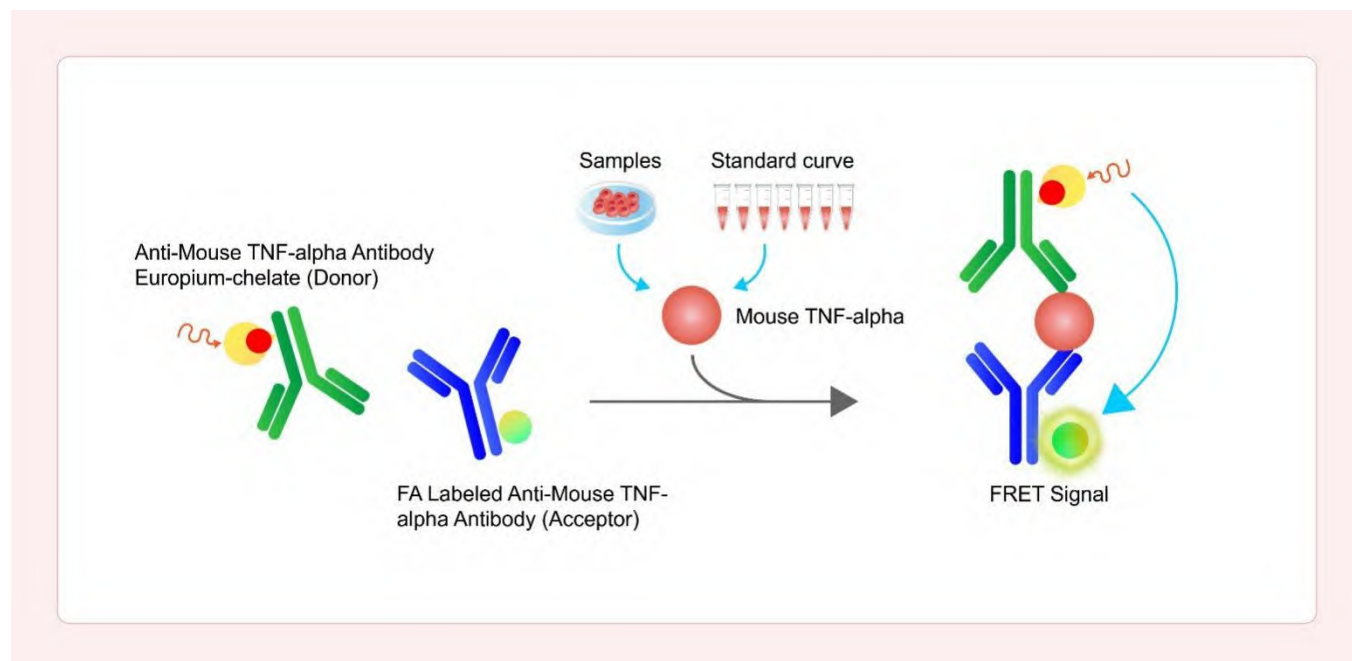
PRODUCT OVERVIEW

Mouse TNF-alpha Detection Kit (TR-FRET) enables sensitive and rapid detection of mouse TNF-alpha in a homogeneous system within 2 hours. Designed for ease of use and high-throughput screening, it is fully compatible with microplate formats, allowing efficient analysis of large sample volumes. This kit is specifically designed for the accurate quantitation of mouse TNF-alpha in cell culture supernatant, serum, and plasma.

PRINCIPLE OF THE ASSAY

This Mouse TNF-alpha 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 mouse TNF-alpha, the Donor and Acceptor are in close proximity because both labeled antibodies bind to the mouse TNF-alpha. 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).

FIGURE 1. PRINCIPLE OF THE ASSAY



MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (100 tests)	Size (500 tests)	Format	Storage	
					Unopened	Opened
FRTB102-C01	Anti-Mouse TNF-alpha Antibody Europium-chelate	100 tests	500 tests	Powder	2-8°C, protected from light	-70°C, protected from light
FRTB102-C02	FA Labeled Anti-Mouse TNF-alpha Antibody	100 tests	500 tests	Powder	2-8°C, protected from light	-70°C, protected from light
FRTB102-C03	Mouse TNF-alpha Standard	5 µg	5 µg	Powder	2-8°C	-70°C
DB-04	TR-FRET Sample Dilution Buffer, pH7.4	50 mL	50 mL	Liquid	2-8°C	2-8°C
DB-05	TR-FRET Detection Buffer, pH7.4	50 mL	50 mL	Liquid	2-8°C	2-8°C

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 μ L, 200 μ L and 1000 μ L 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)
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-Mouse TNF-alpha Antibody Europium-chelate and the FA Labeled Anti-Mouse TNF-alpha 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.	
FRTB102-C01	Anti-Mouse TNF-alpha Antibody Europium-chelate	100 tests	60 µL water	500 tests	300 µL water	/
FRTB102-C02	FA Labeled Anti-Mouse TNF-alpha Antibody	100 tests	60 µL water	500 tests	300 µL water	/
FRTB102-C03	Mouse TNF-alpha Standard	5 µg	50 µL water	5 µg	50 µL water	100 µg/mL

QUICK GUIDE

FIGURE 2. QUICK GUIDE OF THE EXPERIMENTAL WORKFLOW



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 8: 5000 pg/mL) must be diluted.
- 1.2 Serially dilute the provided **Mouse TNF-alpha Standard (FRTB102-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 3.

Note:

1. *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.*
2. *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.*
3. *Do not use TR-FRET Detection Buffer, pH7.4 (DB-05) or any buffers from external sources.*

FIGURE 3. PREPARATION OF SERIAL DILUTIONS OF THE MOUSE TNF-ALPHA STANDARD

Tubes/ Solution Code	Mouse TNF- alpha 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		2 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	
Solution Conc.	100 µg/mL	1 µg/mL	5000 pg/mL	2500 pg/mL	1250 pg/mL	625 pg/mL	312.5 pg/mL	156.25 pg/mL	78.125 pg/mL	39.063 pg/mL	0 pg/mL
Sample Dilution Buffer Vol.		198 µL	398 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL

2. Preparation of Donor working solution

Dilute the Anti-Mouse TNF-alpha Antibody Europium-chelate (FRTB102-C01) stock solution 4-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. Preparation of Acceptor working solution

Dilute the FA Labeled Anti-Mouse TNF-alpha Antibody (FRTB102-C02) stock solution 4-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.

4. Preparation of the pre-mix solution.

Mix the Donor and Acceptor working solution 1:1 (v/v).

5. Addition of all components to the plate

5.1 Add 16 μ L of diluted samples and serially diluted standards to the designated wells according to our recommendation (Table 4) or your own plate layout. Then add 4 μ L of the pre-mix Donor & Acceptor solution to each well.

5.2 Seal the plate with a microplate sealing film and incubate at room temperature (20°C -25°C) for 2 hours on an orbital shaker at 400-600 rpm to ensure the samples, Donor and Acceptor can react adequately.

Refer to Figure 4 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	16 µL Std 8 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Std 8 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Sample Dilution Buffer 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Sample Dilution Buffer 4 µL pre-mixed mouse TNF- alpha antibodies
B	16 µL Std 7 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Std 7 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Sample1 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Sample1 4 µL pre-mixed mouse TNF- alpha antibodies
C	16 µL Std 6 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Std 6 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Sample2 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Sample2 4 µL pre-mixed mouse TNF- alpha antibodies
D	16 µL Std 5 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Std 5 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Sample... 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Sample... 4 µL pre-mixed mouse TNF- alpha antibodies
E	16 µL Std 4 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Std 4 4 µL pre-mixed mouse TNF- alpha antibodies	...	...
F	16 µL Std 3 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Std 3 4 µL pre-mixed mouse TNF- alpha antibodies	...	...
G	16 µL Std 2 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Std 2 4 µL pre-mixed mouse TNF- alpha antibodies	...	...
H	16 µL Std 1 4 µL pre-mixed mouse TNF- alpha antibodies	16 µL Std 1 4 µL pre-mixed mouse TNF- alpha antibodies	...	...

FIGURE 4. PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 8	Std 8	Blank	Blank	...	...	...	...	...	...	...	...
B	Std 7	Std 7	Sample1	Sample1	...	...	...	...	...	...	...	...
C	Std 6	Std 6	Sample2	Sample2	...	...	...	...	...	...	...	...
D	Std 5	Std 5	Sample...	Sample...	...	...	...	...	...	...	...	...
E	Std 4	Std 4	...	...	...	...	...	...	...	...	...	...
F	Std 3	Std 3	...	...	...	...	...	...	...	...	...	...
G	Std 2	Std 2	...	...	...	...	...	...	...	...	...	...
H	Std 1	Std 1	...	...	...	...	...	...	...	...	...	...

6. 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 and subtract average zero standard Ratio to obtain the Δ Ratio.

3. 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: $R^2 \geq 0.9900$.

4. Detection range: 39.063 pg/mL-5000 pg/mL. In this assay, the Δ Ratio value is directly proportional to the analyte concentration. If the Δ Ratio of a test sample is higher than the Ratio corresponding to the 5000 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 the Δ Ratio corresponding to the 39.063 pg/mL standard, the analyte concentration is below the lower limit of quantification and should be reported as < 39.063 pg/mL.

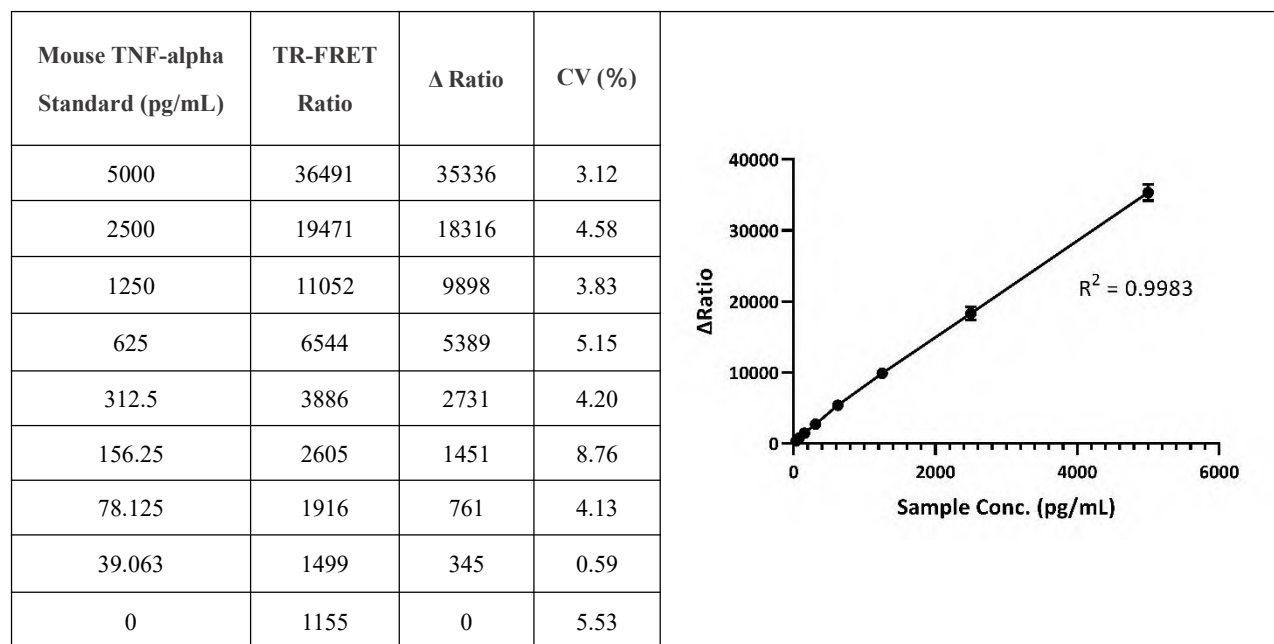
PRECAUTIONS

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°C-25°C) before use. If crystals form in any buffer solution, incubate the solution in a 37°C-40°C 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 Detection Buffer. Do not dilute Donor or Acceptor reagents with the Sample Dilution Buffer.

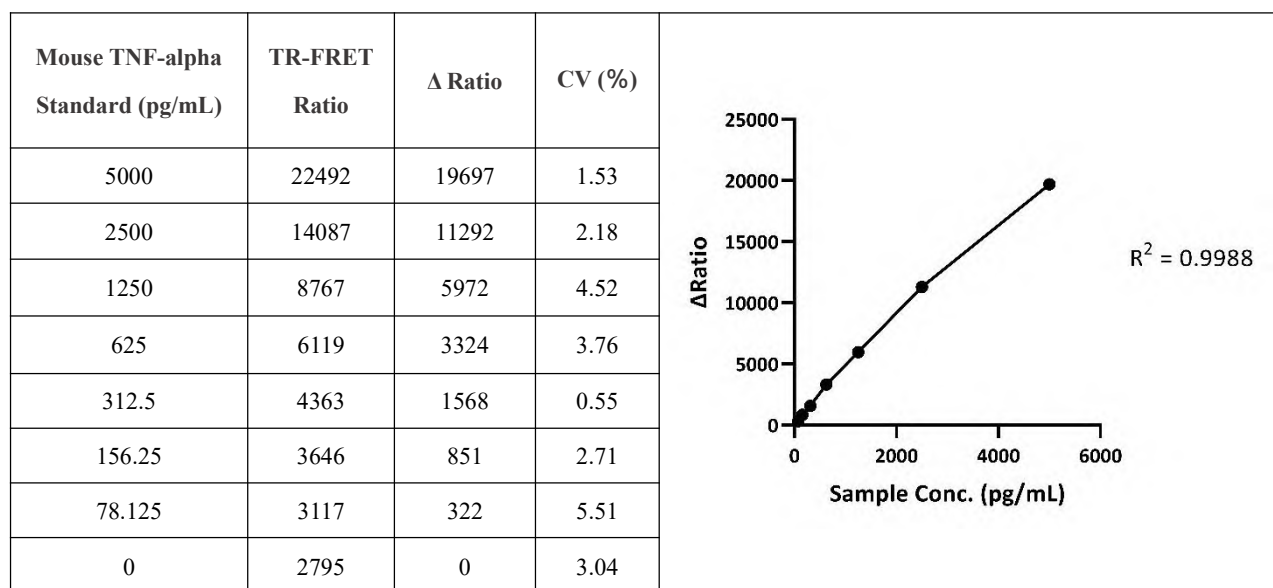
TYPICAL DATA

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

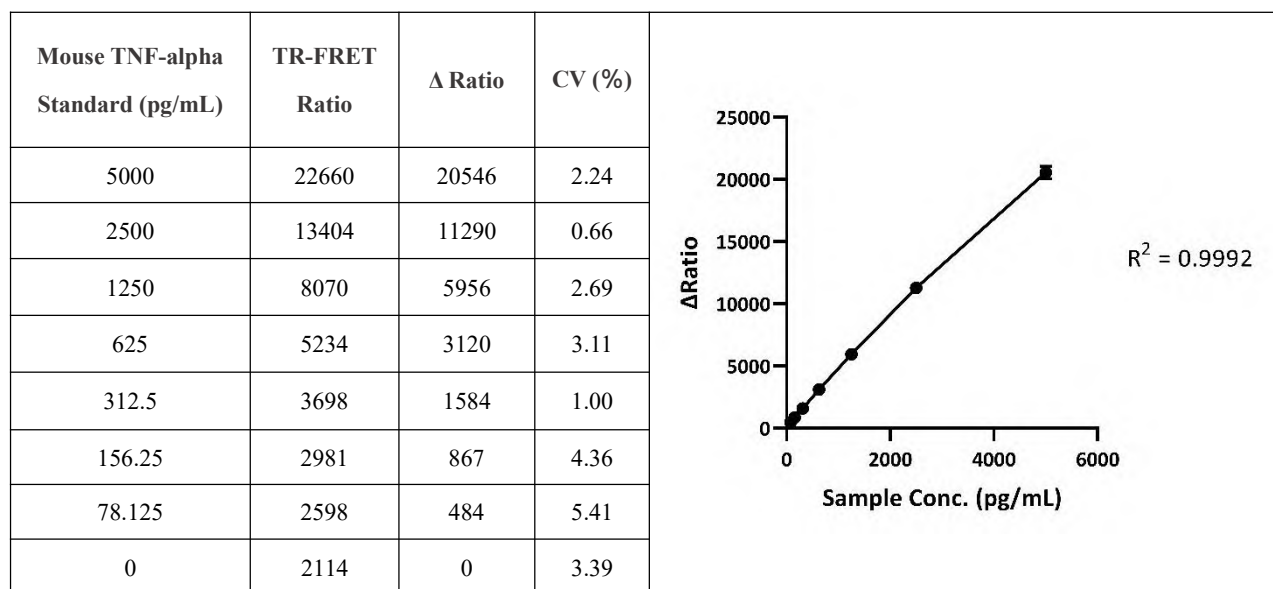
Standard curve generated in TR-FRET Sample Dilution Buffer, pH7.4 (DB-04):



Standard curve generated in DMEM with 10% FBS:



Standard curve generated in RPMI 1640 with 10% FBS:



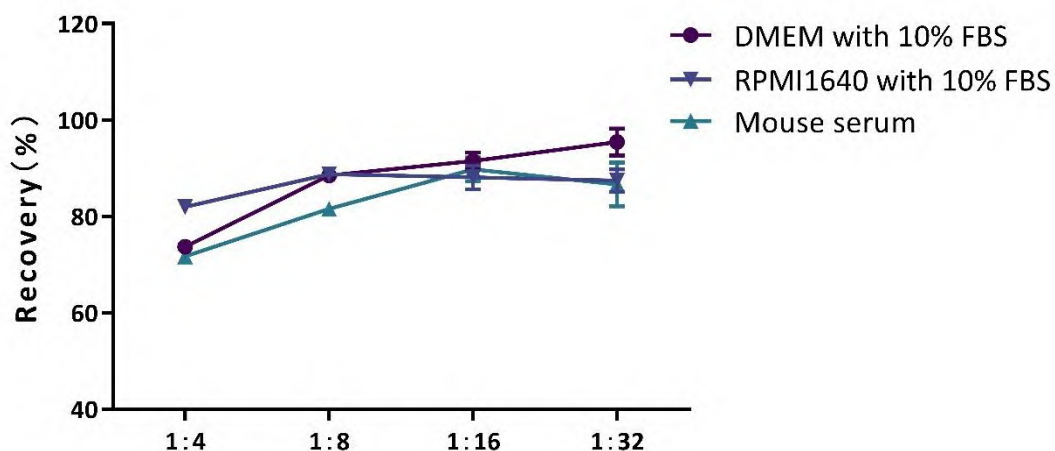
SENSITIVITY

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	39.063 pg/mL-5000 pg/mL	78.125 pg/mL-5000 pg/mL	78.125 pg/mL-5000 pg/mL
Limit of detection (LOD)	43.03 pg/mL	107.80 pg/mL	97.00 pg/mL
Limit of Quantitation (LOQ)	39.063 pg/mL	78.125 pg/mL	78.125 pg/mL

LINEARITY

To evaluate the **linearity** of the assay, samples spiked with high-concentration mouse TNF-alpha 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.



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)	5000	3750	500	80	39.063	5000	3750	500	80	39.063
Number of Replicate	16	16	16	16	16	3	3	3	3	3
Mean (pg/mL)	4579	3598	535	77	37	4558	3510	503	77	38
Standard Deviation	60.17	57.38	21.05	11.85	9.92	127.89	91.42	30.2	3.79	2.95
CV (%)	1.31	1.59	3.94	15.43	26.59	2.81	2.6	6.01	4.93	7.77
Recovery (%)	91.59	95.94	106.95	96.01	95.67	91.16	93.6	100.59	95.94	97.23

CALIBRATION

This immunoassay is calibrated against a highly purified HEK293 cell-expressed recombinant mouse TNF-alpha produced at ACROBiosystems. To convert sample values obtained with the Mouse TNF-alpha Detection kit (TR-FRET) to approximate NIBSC (88/532) nominally assigned mass values, use the equation below.

$$\text{NIBSC/WHO (88/532) approximate value (IU/mL)} = 0.52 \times \text{ACRO Mouse TNF-alpha (pg/mL)}.$$

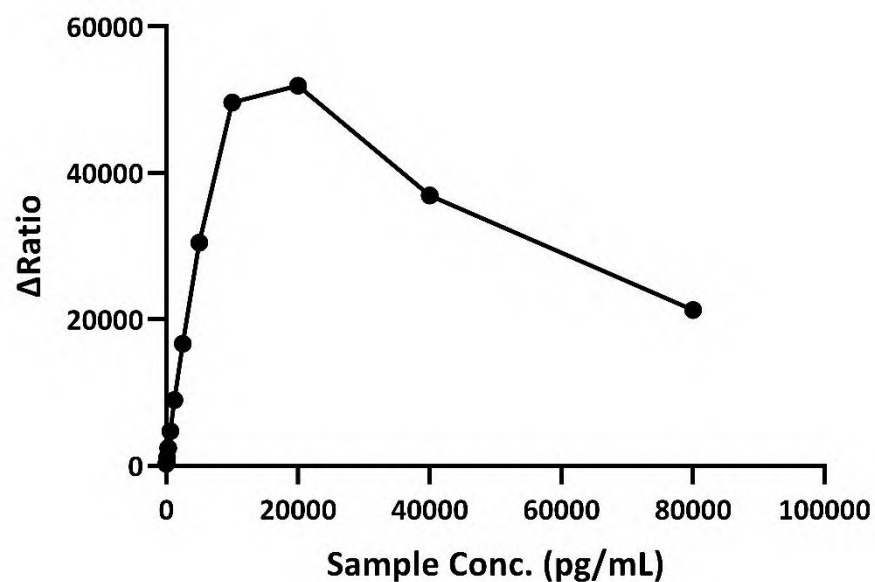
SPECIFICITY

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
IL-2 R alpha	IL-2 R alpha
IL-2 R beta	IL-2 R beta
IL-2 R gamma	IL-2 R gamma
IFN-gamma	IFN-gamma
IL-1 beta	IL-1 beta
TNF-alpha	

Hook Effect

Not be affected by the concentration of Mouse TNF-alpha Standard up to 20000 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.