Human TNFα TR-FRET Detection Kit

Instructions for Use | Cat. No.: ARK0223

1. Principle

This kit quantitatively detects Human TNFα in samples such as cell culture supernatant. It is a sandwich immunoassay based on TR-FRET (time-resolved fluorescence resonance energy transfer). A pair of specific antibodies is labeled with the fluorescent donor Solar Eu and the fluorescent acceptor LA, respectively. When both antibodies bind to different epitopes of Human TNFα, excitation of the donor triggers energy transfer to the acceptor; the signal at 665 nm is proportional to the amount of Human TNFα in the sample.



Figure 1. Principle of the TR-FRET cytokine detection. Donor: Solar Eu; acceptor: LA; Ag: cytokine.

2. Components

Component	Storage	100 tests	1,000 tests	10,000 tests
mAb anti-TNFα-Solar Eu (400X)	⩽ -60 °C	1×5 µL	1×50 µL	1×500 µL
mAb anti-TNFα- LA (400X)	⩽ -60 °C	1×5 µL	1×50 µL	1×500 µL
Human TNFα Standard (25 ng/mL)	⩽ -60 °C	1×50 µL	2 vials (lyo)	2 vials (lyo)
Cytokine Diluent Buffer (ready-to-use)	2–8 °C	2×2 mL	1×20 mL	1×50 mL
Cytokine Detection Buffer (ready-to-use)	2–8 °C	1×2 mL	1×10 mL	1×50 mL

* Test number (tests) refers to the number of wells that can be run in a 96- or 384-well plate with a 20 µL total reaction volume at the recommended reagent concentrations. 400X is the stock concentration; the final concentration in the 20 µL reaction is 1X.

3. Storage

- Store all components under the conditions indicated on their labels. Product quality is guaranteed for 1 year from the date of receipt.
- Human TNFα Standard must be stored at ⩽ -60 °C; the detection antibodies must be stored at ⩽ -60 °C. Aliquot on first use and avoid repeated freeze-thaw cycles; aliquot volumes of at least 10 µL are recommended.

4. Materials Required but Not Provided

Material	Recommended source
Low-volume white 96-well microplate	AREX Biosciences
Low-volume white 384-well microplate	AREX Biosciences
Transparent pressure-sensitive plate seal (readable without removal)	AREX Biosciences
Microplate reader with TR-FRET capability	Various

5. Assay Procedure

5.1 Reaction System

Step	Volume (total 20 µL)	Preparation
1. Sample	16 µL	Test sample or working standard
2. Detection reagent	4 µL	5X premixed detection antibody pair

* The 20 µL system is intended for low-volume 384-well plates. Volumes may be scaled proportionally for 96-well or 1536-well plates.

5.2 Reagent Preparation

- Equilibrate all reagents to room temperature before use and mix liquid reagents gently.
- Reagents stored at ⩽ -60 °C should be thawed on ice and equilibrated to room temperature before use.
- Dilute and prepare all reagents with the buffers supplied in the kit.
- Prepare all working solutions immediately before use.
- Gently rock all reagents to mix. Do not vortex.

5.3 Working Solution Preparation

Standard curve. Prepare the standard dilution series with Cytokine Diluent Buffer or with a solution of the same matrix as the test samples. Using the sample matrix for the standard curve is recommended to reduce matrix effects. The volumes below are for reference only.

Standard	Working conc. (pg/mL)	Final conc. in 20 µL (pg/mL)	Preparation
STD-7	5,000.0	4,000.0	40 µL standard stock + 160 µL Diluent Buffer
STD-6	2,272.7	1,818.2	100 µL STD-7 + 120 µL Diluent Buffer
STD-5	1,033.1	826.4	100 µL STD-6 + 120 µL Diluent Buffer
STD-4	469.6	375.7	100 µL STD-5 + 120 µL Diluent Buffer
STD-3	213.4	170.8	100 µL STD-4 + 120 µL Diluent Buffer
STD-2	97.0	77.6	100 µL STD-3 + 120 µL Diluent Buffer
STD-1	44.1	35.3	100 µL STD-2 + 120 µL Diluent Buffer
STD-0	0	0.0	120 µL Diluent Buffer

Sample preparation. Dilute samples with Cytokine Diluent Buffer or the same matrix solution into the quantifiable range (30 – 5,000 pg/mL). Use the same diluent for samples and standards.

Detection antibodies. Dilute mAb anti-TNFα- Solar Eu (400X) and mAb anti-TNFα- LA (400X) each 40-fold with Cytokine Detection Buffer to obtain the working solution (2 µL per well). Mix equal volumes of the two working solutions to prepare the 5X detection antibody pair premix (4 µL per well).

5.4 Assay Procedure

Step	Negative control	Standard curve	Sample
1	16 µL Diluent Buffer	16 µL diluted standard	16 µL prepared sample
2	Add 4 µL of the 5X detection antibody pair premix to each well		
3	Seal the plate with a transparent pressure-sensitive film and incubate at room temperature for 1–3 hours at room temperature		
4	Read the plate on a TR-FRET compatible microplate reader without removing the film		

5.5 Data Processing

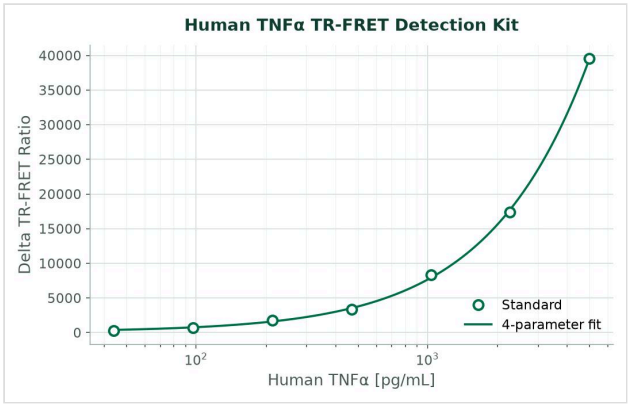
Calculate the TR-FRET ratio and the percentage CV (CV%) for each well:

TR-FRET Ratio = Signal 665 nm / Signal 620 nm × 10,000

6. Results

6.1 Standard Curve

Standard	Concentration (pg/mL)	TR-FRET Ratio	Delta Ratio	CV%
STD-7	5,000.0	40,879	39,532	0.4
STD-6	2,272.7	18,761	17,414	0.5
STD-5	1,033.1	9,674	8,327	2.9
STD-4	469.6	4,732	3,385	2.4
STD-3	213.4	3,121	1,774	3.4
STD-2	97.0	2,017	670	2.3
STD-1	44.1	1,634	287	0.5
STD-0	0	1,347	0	0.6



6.2 Performance

Assay range: 30 – 5,000 pg/mL
Incubation: 3 hours at room temperature

* Sensitivity data were obtained on the specified reader under optimized settings; results may vary with instrument performance.

6.3 Instrument Settings

Vendor	TECAN
Instrument model	Infinite® 200 PRO
Mode	Fluorescence Top Reading
Excitation filter	320 (25) nm
Emission filter 1	665 (8.5) nm
Emission filter 2	620 (10) nm
Mirror	Dichroic 510
Lag time	150 µs
Integration time	500 µs
Number of reads	5 or user-defined
Gain	150 or optimal
Z-focus (mm)	Calculated on the well with the highest signal