Add&Read Human TNF alpha Quantitative Detection Kit(Customized)

DD2704-C

Vazyme

Instruction for Use

Version 24.2

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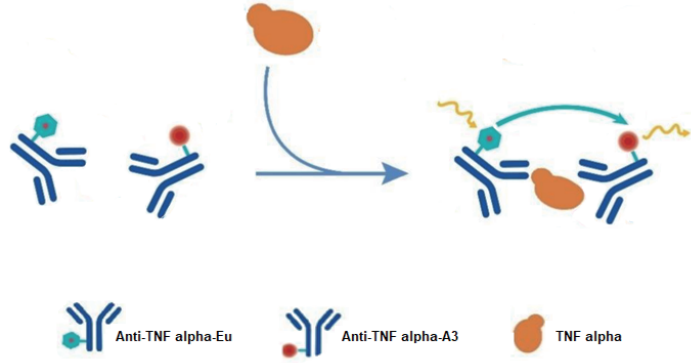
For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

Tumor Necrosis Factor Alpha (TNF alpha) is a pro-inflammatory cytokine mainly produced by macrophages and monocytes. It can directly kill tumor cells without significant toxicity to normal cells. TNF alpha assembles intracellularly to form a non-covalently linked homotrimer and is expressed on the cell surface. It plays a role in normal inflammatory and immune responses by coordinating tissue homeostasis through the regulation of other cytokines' production, cell survival, and death.

This reagent kit utilizes a sandwich method to detect TNF alpha levels. The kit contains TNF alpha Standard and two monoclonal antibodies specific to TNF alpha, one coupled with Eu (donor, Anti-TNF alpha-Eu) and the other coupled with A3 (acceptor, Anti-TNF alpha-A3). When both antibodies bind to TNF alpha simultaneously, Anti-TNF alpha-Eu and Anti-TNF alpha-A3 come close together, leading to fluorescence resonance energy transfer (FRET). Use 320/340 nm excitation light to excite the fluorescent donor, which emits 620 nm light. The 620 nm light excites the fluorescent receptor, which emits 665 nm light. The TNF alpha concentration in the sample is proportional to the FRET signal (ratio of fluorescence intensity at 665 nm to 620 nm).



02/Product Components

Components	DD2704-C-01(96 tests)	DD2704-C-02(500 tests)	DD2704-C-03(10,000 tests)
TNF alpha Standard (Lyophilized)	1 Vial	2 Vial	4 Vial
Anti-TNF alpha-Eu (20 ×)	12 µl	50 µl	1 ml
Anti-TNF alpha-A3 (20 ×)	12 µl	50 µl	1 ml
Detection Buffer(ready-to-use)	500 µl	3 ml	50 ml
Diluent Buffer(ready-to-use)	2 × 1 ml	5 ml	100 ml

03/Storage Conditions

The reagent kit should be stored at -30 ~ -15℃ and transported at ≤0℃.

04/Scope of Application

Cellular supernatant;

05/Self-provided Materials

96/384-well low volume white plate

Microplate reader (with HTRF/TR-FRET module)

06/Precautions

1. Anti-TNF alpha-Eu (20 ×) and Anti-TNF alpha-A3 (20 ×) are recommended to be aliquoted and stored at -30 ~ -15℃ in the stock solution (20 ×), avoiding repeated freeze-thaw cycles. The aliquot volume is suggested to be more than 10 μl.
2. The reconstituted TNF alpha Std is recommended to be stored at -85 ~ -65℃, avoiding repeated freeze-thaw cycles.
3. If slight precipitation occurs in the Detection Buffer and Diluent Buffer, it is considered normal. The precipatation could be gently vortexed at room temperature or a 37℃ water bath to dissolve it before normal use. Thawed Detection Buffer and Diluent Buffer can be stored at 2 ~ 8℃.
4. To check for potential interference effects in your detection buffer during the first use of this reagent kit, we recommend preparing calibration curves in parallel using your own culture medium and diluent.
5. Avoid bubble formation when adding samples.

07/Experiment Process

07-1/Reagent Preparation

1. Preparation of Anti-TNF alpha-Eu and Anti-TNF alpha-A3 working solutions (stock solution is 20 ×)

   For a reaction volume of 20 μl in a 96/384-well low volume white plate, it is recommended to add 2 μl of Anti-TNF alpha-Eu and 2 μl of Anti-TNF alpha-A3 working solutions to each 20 μl system. Before preparing, calculate the required volumes of Anti-TNF alpha-Eu (20 ×) and Anti-TNF alpha-A3 (20 ×) as follows: V = (number of sample wells × 2/20) μl.

   ▲ When calculating the number of sampling wells, the pipetting loss should be taken into account. Generally recommended: Number of sampling wells = Actual number of detection wells × 110%.

   Preparation of Anti-TNF alpha-Eu working solution:

   ● Remove Anti-TNF alpha-Eu (20 ×) from the refrigerator and allow it to thaw at room temperature until completely dissolved. Mix thoroughly before use.

   ● Take 1 volume of Anti-TNF alpha-Eu (20 ×) (1V μl) and add it to 19 volumes of Detection Buffer (19V μl). Mix well and set aside.

Preparation of Anti-TNF alpha-A3 working solution:

● Remove Anti-TNF alpha-A3 (20 ×) from the refrigerator and allow it to thaw at room temperature until completely dissolved. Mix thoroughly before use.

● Take 1 volume of Anti-TNF alpha-A3 (20 ×) (1V μl) and add it to 19 volumes of Detection Buffer (19V μl). Mix well and set aside.

▲ Anti-TNF alpha-Eu (20 ×) and Anti-TNF alpha-A3 (20 ×) are recommended to be aliquoted and stored at -30℃ ~ -15℃, avoiding repeated freeze-thaw cycles.

2. Standard Preparation

The reaction system for a 96/384-well low volume white plate is 20 μl per well, and each well requires 16 μl Standard. Calculate the required Standard volume before preparation.

● Remove the TNF alpha Standard (Lyophilized) from the refrigerator and allow it to equilibrate to room temperature. Reconstitute the TNF alpha Standard (Lyophilized) by adding 400 μl of deionized or distilled water to fully dissolve it, obtaining the TNF alpha Standard.

● You can refer to the table below for Standard gradient dilution. The volumes listed in the table are for dispensing Diluent Buffer.

● Take 60 μl of the reconstituted TNF alpha Std and add it to 120 μl of Diluent Buffer. Mix thoroughly to obtain Std 7.

● Take 60 μl Std 7 and add it to 60 μl of Diluent Buffer. Mix thoroughly to obtain Std 6.

● Dilute 2 times in the same manner to obtain Std 5 to Std 1.

Standard	Dilution Method	Standard Concentration pg/ml
Std 7	60 μl TNF alpha Std + 120 μl Diluent Buffer	2500
Std 6	60 μl Std 7 + 60 μl Diluent Buffer	1250
Std 5	60 μl Std 6 + 60 μl Diluent Buffer	625
Std 4	60 μl Std 5 + 60 μl Diluent Buffer	313
Std 3	60 μl Std 4 + 60 μl Diluent Buffer	156
Std 2	60 μl Std 3 + 60 μl Diluent Buffer	78
Std 1	60 μl Std 2 + 60 μl Diluent Buffer	39
Std 0	60 μl Diluent Buffer	0

▲ The reconstituted TNF alpha Std is stored at -85 ~ -65℃, avoiding repeated freeze-thaw cycles.

07-2/Sample Preparation

To mitigate the effects of matrix interference in samples, it is recommended to dilute the sample with Diluent Buffer at a dilution factor ≥ 2-fold. The specific dilution factor should be determined based on the actual application requirements.

▲ If the sample is diluted with a culture medium, then corresponding calibration curve should also be prepared with the same culture medium.

07-3/Reaction system

1. Sample adding

The reaction volume for the 96/384-well low volume white plate is 20 μl. Adding sample is performed according to the experimental grouping and reaction system outlined in the table below.

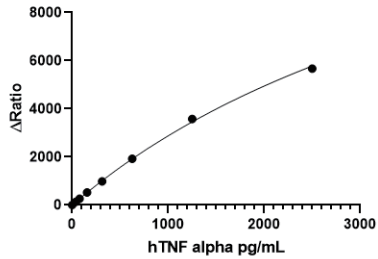
	Standard / Samples	Negative Control
Standard / Samples	16 μl	-
Anti-TNF alpha-Eu	2 μl	2 μl
Anti-TNF alpha-A3	2 μl	2 μl
Diluent Buffer	-	16 μl
Detection Buffer	-	-

2. The order of reagent adding is:
   - Add 16 μl of Standard/sample to the reaction system (96/384-well low volume white plate).
   - Mix the Anti-TNF alpha-Eu working solution and the Anti-TNF alpha-A3 working solution in a 1:1 volume ratio. Then add 4 μl of the mixed solution into the reaction system. It is recommended to gently pipette and mix thoroughly in each well solution gently at least twice.
3. Incubate at room temperature or 25℃ for 2 hours, then detected by an microplate reader (with HTRF/TR-FRET module). The excitation light is 320/340 nm, and emission light at two wavelengths (665 nm and 620 nm) are detected.

08/Data Processing

1. Divide the 665 nm fluorescence value by the 620 nm fluorescence value, then multiply the result by 10^4 to obtain the Ratio value ($665/620*10^4$)
2. Subtract the Ratio value of Standard 0 well from the Ratio value of each well to obtain the ΔRatio value.
3. Perform a 4-parameter fit (weighted $1/y^2$) with TNF alpha concentration as the x-axis and ΔRatio values as the y-axis.
   ▲ Add a weight of $1/y^2$ to the equation to weight the data, ultimately resulting in a 4PL $1/y^2$ fit. The $1/y^2$ weighting correction taken into account is for the variance change that occurs with increasing signal, thereby improving the accuracy of the calibration curve at low/high concentrations.
4. Substitute the ΔRatio value of the sample into the fitting equation of the calibration curve to calculate the sample concentration. Multiply by the dilution factor to obtain the actual concentration of the sample. If the ΔRatio value of the sample exceeds the range of ΔRatio values of the calibration curve, adjust the dilution factor before conducting the detection. This calibration curve is only for demonstration, and a new calibration curve will be generated during each experiment.

	TNF alpha (pg/ml)	Ratio	ΔRatio	CV (%)
Standard 7	2500	5835	5668	2%
Standard 6	1250	3746	3579	2%
Standard 5	625	2093	1926	3%
Standard 4	313	1148	981	1%
Standard 3	156	688	521	2%
Standard 2	78	428	261	3%
Standard 1	39	302	135	2%
Standard 0	0	167	0	0%



09/Product Performance Indexes

09-1/Analytical Sensitivity

Repeat the measurement of Standard 0 twenty times to determine the detection limit, and repeat the measurement of Standard 1 twenty times to determine the quantification limit.

	Diluent	DMEM	RPMI
Limit of detection (LOD)	2 pg/ml	5 pg/ml	5 pg/ml
Limit of Quantitation (LOQ)	39 pg/ml		

09-2/Precision

The reagent kit utilizes five quality control samples of known concentrations add repeated three times on the same ELISA plate to assess within-batch precision. Additionally, five known concentrations of quality control samples are measured by two technicians across six independent analytical batches to evaluate between-batch precision.

Within-batch Precision

Samples	n	Measured average concentration (pg/ml)	SD	CV
Quantitative upper limit concentration sample	3	2343	186	8%
High-concentration sample	3	1933	167	9%
Medium-concentration sample	3	1263	54	4%
Low-concentration sample	3	105	5	5%
Quantitative lower limit concentration sample	3	38	2	7%

Between-batch Precision

Samples	n	Measured average concentration (pg/ml)	SD	CV
Quantitative upper limit concentration sample	6	2404	157	7%
High-concentration sample	6	1976	150	8%
Medium-concentration sample	6	1270	50	4%
Low-concentration sample	6	106	6	6%
Quantitative lower limit concentration sample	6	39	3	7%

09-3/Accuracy

The reagent kit utilizes five known concentrations of quality control samples to repeat the determination three times on the same elisa plate to evaluate the accuracy of the determination concentration to the theoretical concentration ratio.

Samples	n	Theoretical Concentration (pg/ml)	Measured Mean Concentration (pg/ml)	Accuracy % (80-120)
Quantitative upper limit concentration sample	2471 2589 2312	2457	98	92-104
High-concentration sample	2055 1712 1998	1992	96	86-103
Medium-concentration sample	1336 1245 1172	1251	100	94-107
Low-concentration sample	100 100 99	100	100	99-100
Quantitative lower limit concentration sample	41 37 36	38	97	92-105

09-4/Specificity

The reagent kit can detect both native and recombinant human IL6. Specificity testing is conducted for the factors listed below, and no significant cross-reactivity is observed.

Human			
IL1 beta	IL8	IL10	IL2
CXCL-10	IFN gamma	GM-CSF	IL6

09-5/Traceability

NIBSC/WHO (12/154) approximate value (IU/ml) = 0.1 × Human TNF alpha value (pg/ml).

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