Mouse TNFα VeriQuant Immunoassay Kit

Catalog NO.: VQ1060M

This manual must be read attentively and completely before using this product. If you encounter any issues, please contact our Technical Support Center for assistance.

Phone: +86-27-65316809
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The shelf life and storage temperature of the kit are indicated on the outer label. Please use the kit within the valid period. Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Product Description

The Mouse TNFα VeriQuant™ Immunoassay Kit is designed for the in vitro quantitative detection of Mouse TNFα concentrations in serum, plasma and other biological fluids using a real-time quantitative PCR (qPCR) instrument.

This protocol describes the qPCR experimental test using **5 µL** sample size and **50 µL** reaction system.

For adjustments to the qPCR reaction system, please consult technical support or refer to www.reedbiotech.com.

Basic Performance

Sensitivity	0.105pg/mL
Detection Range	0.64-10000 pg/mL

Test Principle

Two antibodies recognizing distinct epitopes of the target protein are each conjugated with specific oligonucleotides. When both antibodies bind to the same target protein, the two oligonucleotides are brought into close proximity. Subsequently, Synthesis Enzymes ligate the oligonucleotides to form a template, which is amplified via qPCR using specific primers and TaqMan probes. Fluorescence signal intensity is negatively correlated with the cycle threshold (Ct) value, enabling high-specificity and high-sensitivity quantitative detection of the target protein.

Kit Components & Storage

An unopened kit can be stored at -20 °C for 12 months. If the opened kit is not used up, store the items separately according to the following conditions.

Item	Quantity
Standard	2 vials
Assay Dilution Buffer	7.5mL
Antibody-conjugate A	15µL
Antibody-conjugate B	15µL
Antibody-conjugate Dilution Buffer	1mL
Quanti Mixture	5mL
Synthesis Enzymes	30µL
Product Manual	1 copy
Certificate of Analysis	1 copy

Materials Required, Not Supplied

- Real-time quantitative PCR instrument
- Two pieces of 96-well 0.2 mL PCR plates (PCR 8-tube strips or single PCR tubes are acceptable alternatives)
- High-precision single-channel and multi-channel pipettes, centrifuge tubes, and low-retention pipette tips (0.5–10 µL, 2–20 µL, 20–200 µL, 200–1000 µL)
- Incubator
- Pressure-sensitive high-adhesion qPCR sealing film, sealing film scraper or roller
- 2 × 5 mL reagent reservoirs
- 96-well low-temperature metal ice block
- DNase/RNase/pyrogen-free 1.5 mL、5mL centrifuge tubes
- Microplate centrifuge

Note

1. Refer to the detailed VeriQuant™ technical guidelines on Reedbiotech.com prior to operation.
2. Wear gloves, use DNase/RNase/pyrogen-free consumables and follow standard molecular laboratory operating procedures.
3. Centrifuge vials before pipetting to ensure the contents are at the bottom of the tube.
4. Thaw all reagents at room temperature except Synthesis Enzymes. Keep Synthesis Enzymes (no thawing required) and all thawed reagents on ice during the entire experiment.
5. Do not vortex the PCR plate.
6. Use a scraper or roller to ensure complete adherence to avoid any evaporation or contamination.
7. Perform standard pipetting to minimize the coefficient of variation (CV).
8. If any particulate matter is present in the sample, centrifuge or filter sample before performing the assay.

Reagents Preparation

1. Create Standard Curve

①Prepare a 10000pg/mL standard working solution immediately by diluting one vial of standard with **1mL** Assay Dilution Buffer. Mix thoroughly by inverting the vial up and down five times; do not vortex.Stand at room temperature for 15 minutes.

②Prepare **PCR Plate 1** (for temporary storage of all prepared working solutions to facilitate subsequent multichannel pipetting; standard curve dilutions must be performed within the same column. Keep PCR Plate 1 on a low-temperature metal ice block throughout the experiment).

③Conduct 5-fold serial dilution: Add **80 µL** of Assay Dilution Buffer to each well starting from the second well of the standard column on PCR Plate 1. Pipette **100 µL** of the 10000pg/mL standard working solution into the first well (marked **S7**). Transfer **20 µL** from S7 to the second well and mix to obtain **S6** (2000pg/mL). Continue the dilution sequentially down to the penultimate well (**S1**). The last well serves as the blank control (**S0**) with no standard added. Prepare standard working solutions freshly right before use.

Standard	Assay Dilution Buffer	Concentration
S7: 100 µL	0 µL	S7: 10000pg/mL
S7: 20 µL	80 µL	S6: 2000pg/mL
S6: 20 µL	80 µL	S5: 400pg/mL
S5: 20 µL	80 µL	S4: 80pg/mL
S4: 20 µL	80 µL	S3: 16pg/mL
S3: 20 µL	80 µL	S2: 3.2pg/mL
S2: 20 µL	80 µL	S1: 0.64pg/mL
N/A	80 µL	S0: 0 pg/mL

2. Sample Dilution 10 Times

Add **5 µL** of sample to **45 µL** of Assay Dilution Buffer in PCR Plate 1, then mix well by pipetting with a pipette.

3. Mix Antibody-Conjugates

①Add the following components into a 1.5 mL centrifuge tube at a volume ratio of **1:1:100**, and mix by inverting the tube up and down.

Component	Volume
Antibody-conjugate A	7 µL
Antibody-conjugate B	7 µL
Antibody-conjugate Dilution Buffer	700 µL

②Add ≥80 µL of the mixed antibody-conjugate mixture to each well of one column on PCR Plate 1.

③Press the sealing film evenly with a scraper or roller for complete sealing. Tap the side of the plate gently three times for thorough mixing, then centrifuge the plate at 3000 g for 1 minute using a microplate centrifuge.

4.Prepare qPCR reaction mixture

Pre-mix qPCR reaction mixture before the completion of Step A: Add **25 µL** of Synthesis Enzymes into the Quanti Mixture tube(The volume in the tube is **5mL**, which should be calculated according to the actual dosage. The volume ratio of synthesis enzymes and quantity mixture is **1:200**). Mix by inverting the tube five times. Transfer the mixture to a reagent reservoir for convenient multi-channel pipetting if needed.

Assay Procedures

A. Analyte Binding

1. Transfer **5 µL** of the antibody-conjugate mixture from PCR Plate 1 to all test wells of **PCR Plate 2** (the final reaction plate) using a multi-channel pipette.
2. Transfer **5 µL** of standard solution or diluted sample from PCR Plate 1 to the corresponding wells of PCR Plate 2, then mix by gentle pipetting. Seal the plate with sealing film, tap the plate side gently to mix, and centrifuge at 3000 g for 1 minute. Incubate the plate at **37 °C for 1 hour.**

B. qPCR Amplification

3. Add **40 µL** qPCR reaction mixture to all detection wells of A-reaction PCR plate 2. Mix gently and avoid bubble formation. Seal the plate tightly with qPCR sealing film and press firmly with a scraper or roller.Tap the plate side gently to mix, then centrifuge at 3000 g for 1 minute.
4. Create a new protocol on the qPCR instrument with the following settings:

Item	Parameter
Assay Type	Standard Curve
Reporter Dye	FAM
Quencher	NFQ-MGB *
Reference Dye	None
Sample Type for all wells	Unknown
Threshold	0.2
Baseline	15

*If the corresponding option is unavailable on your instrument, select "None" or "Non-fluorescent".

5. Thermal cycling parameters:

Step	Temp(°C)*	Time	Stage
Ligation	25	20min	Hold
Inactivation	95	2min	Hold
Denaturation	95	15s	40 cycles
Annealing/extension	60	1min	

Ramp rate: **1.6 °C/s**

6. Save the protocol as a template and start the assay. Reuse the saved template for subsequent assays.

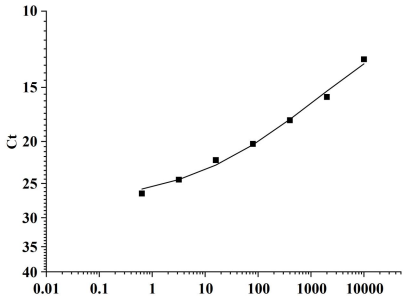
Data Analysis

1. Save the assay data in .ed, .sds or .csv format.
2. Calculate the average Ct value for duplicate wells of each standard. Use the 4-parameter logistic (4PL) curve fitting tool (available on www.reedbiotech.com) to generate a standard curve on a double logarithmic coordinate system, with standard concentration on the X-axis and average Ct value on the Y-axis. Calculate sample concentrations by substituting sample average Ct values into the standard curve equation.

Example of Extended Standard Curve

The data in this section is provided as an example of a typical standard curve. Prepare your own standard curve for each plate.

Concentration (pg/mL)	Average Ct
10000	12.921
2000	15.787
400	17.867
80	20.257
16	22.1
3.2	24.509
0.64	26.382
0	27.605



Disclaimer

1. Due to current technical limitations, comprehensive identification of all raw materials cannot be guaranteed. Potential quality and technical risks of this product exist.
2. This kit is optimized to reduce endogenous interfering factors in biological samples, while not all potential interfering substances can be eliminated.
3. Experimental results are affected by reagent performance, operator proficiency and experimental environment. The manufacturer is only responsible for the quality of the kit itself.
4. For reliable results, only use reagents supplied in this kit. Do not mix with products from other manufacturers and strictly follow this manual.
5. Improper reagent preparation or qPCR instrument parameter settings may lead to abnormal results. Please read the manual thoroughly and set up the instrument correctly before use.
6. Variations may exist between independent tests even when performed by the same operator. Standardize all operating steps to ensure result reproducibility.
7. All kits undergo strict quality inspection before delivery. Discrepancies between test results and factory data may be caused by transportation conditions, instrument differences and batch variations.
8. This kit has not been compared with similar products or detection methods from other manufacturers, so inconsistent test results may occur.
9. This kit is intended for research use only. The manufacturer shall not be liable for any consequences arising from use for clinical diagnosis or other unauthorized purposes.