

ProQuantum™ Human TNFα Immunoassay Kit

Catalog Number A35601 (96 reactions), A356015 (5 × 96 reactions)

Pub. No. MAN0018209 Rev. B.0

Product description

The ProQuantum™ Human TNFα Immunoassay Kit is designed to quantify human TNFα protein in serum, plasma, and cell culture supernatant using a qPCR instrument. The assay is expected to work with other sample types, but may need protocol modifications for optimal results. This protocol describes how to run an assay using an entire 96-well plate with 5-μL sample volumes. To customize plate design, or for protocols using samples at half volume (2 μL), go to apps.thermofisher.com/apps/proquantum.

Contents and storage

Upon receipt, store the kit at -20°C. Expires one year from date of receipt when stored as instructed.

| Contents | Cat. No. A35601 (96 rxn) |
|--|--------------------------|
| Human TNFα Antibody-conjugate A | 15 μL |
| Human TNFα Antibody-conjugate B | 15 μL |
| Human TNFα Protein Standard, lyophilized | 2 vials |
| Master Mix | 5 mL |
| Ligase | 40 μL |
| Antibody-conjugate Dilution Buffer | 700 μL |
| Assay Dilution Buffer | 7.5 mL |

General guidelines

- Review the *ProQuantum™ Technical Guide* at thermofisher.com for details before starting the procedure.
- Wear gloves, use DNase/RNase/pyrogen free plastic ware, and practice proper RNA handling techniques.
- Centrifuge vials before pipetting to ensure the contents are at the bottom of the tube.
- Thaw all reagents **except Ligase** at room temperature. Keep Ligase and thawed reagents on ice.
- Do not vortex plates.
- Use a plate seal hand tool to ensure complete adherence to avoid any evaporation or contamination.
- Use best practices with pipetting to minimize CV.
- If any particulate matter is present in the sample, centrifuge or filter sample before performing the assay.

Required materials not provided

| Product | Cat. No. [1] |
|---|-------------------|
| Calibrated pipettes and low retention filter tips | MLS |
| 96-well plate cold block | Fisher 50-589-601 |
| 96-well assay plate and 96-well working plate | MLS |
| 25-mL reagent reservoir × 2 | Fisher 14387071 |
| Microtiter plate adhesive seals × 2 | Fisher 4306311 |
| Microtiter plate adhesive seal, optical grade | Fisher AB1170 |
| Plate seal hand tool | 4333183 |
| Centrifuge with swinging bucket rotor for 96-well plates, and microcentrifuge | MLS |
| 96-well plate rack | Fisher 05-541-80 |
| RNase-free 1.5-mL microcentrifuge tube | AM12400 |
| Sterile 15-mL conical tube | 339651 |

[1] MLS: major laboratory supplier.

Prepare reagents

Reconstitute standard

- Reconstitute one vial of protein standard with Assay Dilution Buffer to 5,000 pg/mL. See label on the vial for reconstitution volume. Mix by inverting the vial five times. **Do not vortex.**
- Incubate for 15 minutes at room temperature.

Prepare working plate (on cold block 4°C)

Mix antibody-conjugates

- Add the following components to a 1.5-mL microcentrifuge tube, then mix by pipetting up and down.

| Component | Volume |
|------------------------------------|--------|
| Antibody-conjugate A | 12 μL |
| Antibody-conjugate B | 12 μL |
| Antibody-conjugate Dilution Buffer | 696 μL |

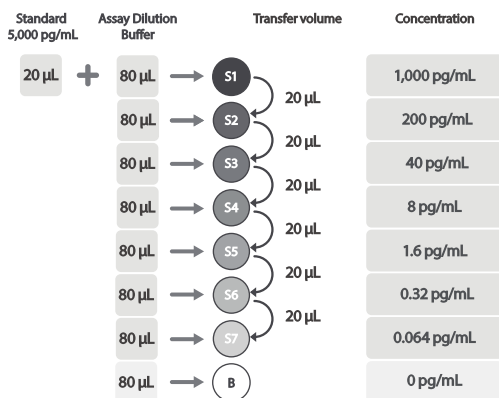
- Dispense ≥80 μL of antibody-conjugate mixture to each well in one column of the working plate.

Dilute samples 10-fold

Add 5 μL of sample to 45 μL of Assay Dilution Buffer in the working plate, then mix by pipetting up and down.

Create standard curve

- Add 80 μL of Assay Dilution Buffer to each well (S1–S7) in one column of the working plate that is reserved for standards.
- Mix reconstituted standard by gently inverting the vial five times. Transfer 20 μL of protein standard to the first well (S1), then mix.
- Make serial dilutions of the standard by transferring 20 μL from well S1 to well S2. Continue serial dilution to well S7. The Background well (B) should only contain Assay Dilution Buffer. Mix wells thoroughly. Change pipette tips between dilution steps.



Note: The standard curve can be modified to extend the range. See “Example of extended standard curve” on page 2.

- Seal the working plate with an adhesive seal using the plate seal hand tool. Mix by striking the side of the plate against the palm of your hand three times. Rotate the plate and strike the other side of the plate against your palm three times.
- Centrifuge at $3,000 \times g$ for 1 minute. Discard remaining unused reconstituted standard.

Run assay

Bind analyte (1 hour)

Keep the assay plate on a plate rack except during mixing or centrifugation steps.

- Transfer 5 μL of antibody-conjugate mixture from the working plate by multichannel pipette to all assay wells in the assay plate.
- Transfer 5 μL of standards or diluted samples from the working plate to the appropriate wells in the assay plate. Mix thoroughly by pipetting up and down several times.
- Seal the assay plate with an adhesive seal. Mix by striking the plate. Centrifuge at $3000 \times g$ for 1 minute.
- Incubate the assay plate for 1 hour at room temperature.
(Optional) Incubate the assay plate overnight at 4°C .

Perform qPCR

- Add 5 mL of Master Mix and 30 μL of Ligase to a 15-mL conical tube. Invert the tube five times, then pour the qPCR reaction mixture into a reagent reservoir.
- Add 40 μL of qPCR reaction mixture to all assay wells. Mix by pipetting up and down. Avoid introducing bubbles in the wells.
- Seal the assay plate with an optical plate seal. Ensure there is complete adhesion using the plate seal hand tool.
- Mix by striking plate, then centrifuge at $3,000 \times g$ for 1 minute.
- Create a new Experiment on the qPCR instrument.

| Parameter | Settings for Applied Biosystems™ instruments |
|-------------------|--|
| Experiment type | Standard Curve or Quantitation - Standard Curve |
| Reagents | TaqMan™ reagents |
| Reporter dye | FAM |
| Quencher | NFQ-MGB [1] |
| Passive reference | ROX |
| Assign wells | Define all wells of the 96-well plate as Unknown |
| Threshold | 0.2 |
| Baseline | 3–15 |

[1] For instruments without this option, enter "None" or "Non-fluorescent".

- Input the PCR Method based on your instrument block type.

| Step | Temp (°C)[1] | Time (by block type) | | Stage |
|---------------------|--------------|----------------------|---------|-----------|
| | | Standard | Fast[2] | |
| Ligation | 25 | 20 min | 20 min | Hold |
| Ligase inactivation | 95 | 2 min | 2 min | Hold |
| Denaturation | 95 | 15 s | 1 s | 40 cycles |
| Annealing/extension | 60 | 1 min | 20 s | |

[1] Set ramp rate to $2^\circ\text{C}/\text{sec}$

[2] Use default values for 7500, 7500 Fast, 7900HT, or non-Applied Biosystems instruments (e.g., 3 s denaturation and 30 s annealing/extension).

- Save the Experiment as a template. Run the template.

Note: Reuse the saved template for subsequent assays.

Import run data

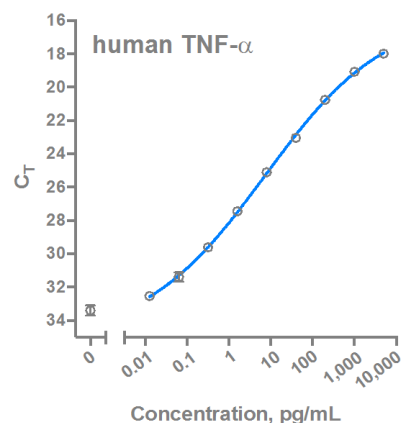
- Save the run data in .eds or .sds format (or .csv format for non-Applied Biosystems instruments) to a USB memory device, or directly to the Connect cloud-based platform.

Note: If your qPCR instrument is connected to a computer, download the run data from the computer and **not** directly from the qPCR instrument.

- Import .eds, .sds, or .csv files to the ProQuantum™ software (available at apps.thermofisher.com/apps/proquantum) to generate standard curves and determine sample concentrations.

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 assay plate.



| Concentration (pg/mL) | Average C_t |
|-----------------------|---------------|
| 5,000 | 18.0 |
| 1,000 | 19.1 |
| 200 | 20.8 |
| 40 | 23.0 |
| 8 | 25.1 |
| 1.6 | 27.4 |
| 0.32 | 29.6 |
| 0.064 | 31.4 |
| 0.0128 | 32.5 |
| 0 | 33.4 |

Limited product warranty

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