

ab309419 – Human TNF-alpha ELISA Kit (For Lysates)

For the quantitative measurement of human TNF-alpha cell lysate and tissue lysates. For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab309419>

Storage and Stability:

The entire ELISA kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. Observe the storage conditions for individual prepared components in the Reagent Preparation.

Materials Supplied

Item	Quantity	Storage Condition
20X Wash Buffer	25 mL	-20°C
2X Lysis Buffer (Item J)	5 mL	-20°C
5X Assay Diluent (Item E2)	15 mL	-20°C
5X Sample Diluent Buffer (Item D2)	15 mL	-20°C
600X HRP-Streptavidin	200 µL	-20°C
Biotinylated Human TNF-alpha Detection Antibody (For Lysates)	2 vials	-20°C
Human TNF-alpha Antibody-coated ELISA Plate (For Lysates)	1 unit	-20°C
Lyophilized Human TNF-alpha Protein Standard (For Lysates)	2 vials	-20°C
Stop Solution	8 mL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µl to 1 ml volumes.
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions
- Log-log graph paper or computer and software for ELISA data analysis.

Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

5X Sample Diluent Buffer (Item D2) and 5X Assay Diluent (Item E2) - Dilute Sample Diluent Buffer (Item D2) and 5X Assay Diluent (Item E2) 5-fold with deionized or distilled water before use. 2X Lysis Buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

Sample dilution: Tissue lysate and cell lysate samples should be diluted at least 5-fold with 1X Sample Diluent Buffer. Generally, we recommend a minimum of 1 mg of protein per 1 ml of original lysate solution, though more concentrated is better. We also recommend the addition of protease inhibitors (not included) to the lysis buffer prior to use.

Note: Levels of TNF-alpha may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Detection Antibody: Briefly spin the vial before use. Add 100 µL of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent.

20X Wash Buffer: If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

600X HRP-Streptavidin: Briefly spin the vial of 600X HRP-Streptavidin concentrate before use. 600X HRP-Streptavidin should be diluted 600-fold with 1X Assay Diluent. For example: Briefly spin the vial and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent to prepare a 600-fold diluted HRP Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Standard Preparation

Always prepare a fresh set of standards for every use.

Discard working standard dilutions after use as they do not store well.

The following section describes the preparation of a standard curve for duplicate measurements (recommended).

- Briefly spin the Standard Protein.
- Add 400 µl of 1X Sample Diluent Buffer (Sample Diluent Buffer should be diluted 5-fold with deionized or distilled water before use) into the standard protein vial to prepare a 60 ng/ml standard. Gently mix the powder to allow it to dissolve thoroughly.
- Add 50 µl TNF-alpha standard from the vial, into a tube with 450 µl Sample Diluent Buffer to prepare a 6000 pg/ml standard solution.
- Pipette 300 µl 1X Sample Diluent Buffer into each tube.
- Use the 6,000 pg/ml standard solution to produce a dilution series. Adding 200 µL from #1 to #2, then from #2 to #3, etc.
- Mix each tube thoroughly before the next transfer.
- Tube #8 contains no protein and is the Blank control.

Tube #	Volume to dilute	Volume of 1X Assay Diluent	Final Concentration pg/mL
1	6000 pg/ml standard stock solution	---	6000
2	200 µL of tube #1	300 µL	2400
3	200 µL of tube #2	300 µL	960
4	200 µL of tube #3	300 µL	384
5	200 µL of tube #4	300 µL	153.6
6	200 µL of tube #5	300 µL	61.44
7	200 µL of tube #6	300 µL	24.58
8	--	300 µL	0

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use. We recommend that you assay all standards, controls and samples in duplicate. Prepare all reagents, working standards, and samples as directed in the previous sections.

1. Label removable 8-well strips as appropriate for your experiment.
2. Add 100 μ L of standard or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature with gentle shaking.
3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 μ L) using a multichannel Pipette or auto washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μ L of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 μ L of prepared Streptavidin solution each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 μ L of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 μ L of Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus