



KAMIYA BIOMEDICAL COMPANY

Human Tumor Necrosis Factor Alpha (TNFA) ELISA

**For the quantitative determination of human TNFA in
biological samples**

Cat. No. KT-53374

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

Product Information
Human Tumor Necrosis Factor Alpha (TNFA) ELISA
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INTENDED USE

This TNFA ELISA kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures. The stop solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of TNFA in the sample, this TNFA ELISA kit includes a set of calibrators. The calibrators are assayed at the same time as the samples and allow the operator to produce a calibration curve of optical density versus TNFA concentration. The concentration of TNFA in the samples is then determined by comparing the O.D. of the samples to the calibration curve.

PRINCIPLE

This kit for the quantitative measurement of TNFA utilizes a polyclonal anti-TNFA antibody and an TNFA-HRP conjugate. The assay sample and buffer are incubated together with TNFA-HRP conjugate are incubated together in the pre-coated plate for one hour. After the incubation period the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reactions, which will then turn the solution yellow. The intensity of the color is measured spectrophotometrically at 450 nm in a microplate reader. The intensity of the color is inversely proportional to the TNFA concentration since TNFA from samples and TNFA-HRP conjugate complete for the anti-TNFA antibody binding site. Since the number of sites is limited, as more sites are occupied by TNFA from the sample, fewer sites are left to bind TNFA-HRP conjugate. Calibrators of known concentrations are run concurrently with the samples being assayed and a calibration curve is plotted relating the intensity of the color (Optical Density) to the concentration of TNFA. The TNFA concentration in each sample is then interpolated from the calibration curve.

COMPONENTS

Reagents	Quantity
Pre-coated 96-well plate	1
Calibrator 1 (0 pg/mL)	1
Calibrator 2 (50 pg/mL)	1
Calibrator 3 (100 pg/mL)	1
Calibrator 4 (250 pg/mL)	1
Calibrator 5 (500 pg/mL)	1
Calibrator 6 (1000 pg/mL)	1
Enzyme Conjugate	1 x 6 mL
Substrate A	1 x 6 mL
Substrate B	1 x 6 mL
Stop Solution	1 x 6 mL
Wash Buffer (100X concentrate)	1 x 10 mL
Lysis Buffer Solution	1 x 6 mL

Note: The lysis buffer solution is used only when the sample is cell culture fluid & body fluid & tissue homogenate; If the sample is serum or blood plasma, then the lysis buffer solution is a superfluous reagent.

STORAGE

All reagents should be stored at 4°C upon receipt. For expiration date refer to kit label.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1,000 x g. Remove serum and assay immediately or store samples in aliquots at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or store samples in aliquots at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Cell Culture Supernates and Other Biological Fluids

Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.



Note:

1. Samples to be used within 7 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. When performing the assay, slowly bring samples to room temperature.
3. Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.
4. Do not use heat treated specimens.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader with 450 nm filter.
2. Precision pipettes to deliver 1-2 mL volumes.
3. Adjustable 10-100 mL pipettes for reagent preparation.
4. 100 mL and 1 L graduated cylinders.
5. Calibrated adjustable precision pipettes with disposable tips (multi-channel is desirable for large assays).
6. 37°C incubator.
7. Absorbent paper.
8. Distilled or deionized water
9. Data analysis tools such as graphing software, or graph paper (linear, log-log, semi-log, or log-logit as desired).
10. Tubes to prepare Calibrators or sample dilutions.

Precautions

1. **Kamiya Biomedical Company** is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assaying. If values for these samples are not within the range of the calibration curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g. antibody targets conformational isotope rather than linear isotope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

5. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Fresh samples without long term storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Bring all kit components and samples to room temperature (18-25°C) before use.

Dispense 10 µL of lysis buffer solution into 100 µL specimens, mix and stand for one hour (The proportion of lysis buffer and specimens shall be no less than 1:10). (**NOTE:** This step is required when the sample is cell culture fluid & body fluid & tissue homogenate; If the sample is serum or blood plasma, then this step should be skipped.)

Wash Solution

Dilute 10 mL of Wash Solution concentrate (10X) with 990 mL of de-ionized or distilled water to prepare 1,000 mL of Wash Solution (1X).

ASSAY PROCEDURE

1. Prepare all calibrators before starting assay procedure (see Reagent Preparation). It is recommended that all calibrators and samples be added in duplicate to the microtiter plate.
2. First, secure the desired number of coated wells in the holder, then add 100 µL of calibrators and samples to the appropriate well of the antibody coated microtiter plate.
3. Add 50 µL of conjugate to each well. Mix well. Complete mixing in this step is important. Cover and incubate for 1 hour at 37°C.
4. Wash the microtiter plate using one of the specified methods indicated below:
5. Manual Washing: Remove incubation mixture by aspiration contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with distilled or de-ionized water, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of five washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.
6. Automated Washing: Aspirate all wells, then wash plate five times using distilled or de-ionized water. Always adjust your washer to aspirate as much liquid as possible and set fill volume to 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper and paper towels until no moisture appears. It is recommended that the washer be set for a soaking time of 15-30 seconds or shaking time of 5 seconds between washes.
7. Add 50 µL substrate A and B to each well. Cover and incubate for 15 minutes at 20-25°C.
8. Add 50 µL stop solution to each well. Mix well.
9. Read the optical density (OD) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

1. This calibration curve is used to determine the amount in an unknown sample. The calibration curve is generated by plotting the average OD (450 nm) obtained for each of the six calibrator concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2. First, calculate the mean OD value for each calibrator and sample. All OD values are subtracted by the mean value of the zero calibrator before result interpretation. Construct the calibration curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the OD value on the Y-axis and extend a horizontal line to the calibration curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in the result. Each user should obtain their own calibration curve.
5. The sensitivity by this assay is 1.0 pg/mL.

REAGENT PREPARATION

All reagents must be allowed to reach room temperature before use. Additional information for individual reagents can be found on vial labels.



Note:

1. This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drain with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin, and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.
2. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

IMPORTANT NOTES

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 4°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin free tubes.
4. Samples should be frozen if not utilized shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to use.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to TNFA lysis.
6. It is recommended that all calibrators, controls, and samples be run in duplicate.
7. When pipetting reagents maintain a consistent order from well-to-well. This ensures equal incubation time for all wells.
8. Cover or cap all reagents when not in use.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. The provided protocols should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into wells.
14. Because stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between stabilized Chromogen and metal, or color may develop.
15. Incomplete washing will adversely affect the test outcome. All washing must be performed with wash buffer provided.
16. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering TNFA aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.
17. After aspiration, fill the wells with at least 0.35 mL of diluted wash solution. Let soak for 15-30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
18. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
19. If using an automated washer, the operating instructions for washing equipment should be carefully followed.
20. Assay Procedure Preliminary notes: Do not mix reagents from different lots. It is recommended that assays be performed in duplicate. Calibrators and samples must be assayed at the same time. Avoid exposing the substrate to direct sunlight.

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