

Human TNF- α ELISA

Cat. No. : KB1145

Ver1,0

ELISA Set for Accurate Quantitation from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

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Introduction:

Tumor Necrosis Factor- α (TNF- α) is a potent lymphoid factor which exerts cytotoxic effects on a wide range of tumor cells and certain other target cells. Human TNF- α is a 17.4 kD protein containing 157 amino acid residues.

Intended Use:

Human TNF- α ELISA is specifically designed for the accurate quantitation of human TNF- α from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

Materials Provided :

1. Microtitre coated plate (96 wells) – 1 no
2. Recombinant human TNF- α Standard, 1 μ g/ml, 20 μ l – 1 vial
3. Human TNF- α Detection Antibody, 100 μ l – 1 vial
4. Concentrated Avidin Horseradish Peroxidase (Av-HRP), 40 μ l - 1 vial
5. 20X Wash Buffer – 20ml
6. 5X Assay Diluent – 15ml
7. TMB Substrate – 12ml
8. Stop Solution – 12ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450nm.
2. Adjustable pipettes to measure volumes ranging from 50 μ l to 1000 μ l.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Log-Log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components at 2-8°C.
2. Store recombinant standard at -20°C. Upon first thaw, aliquot recombinant protein into polypropylene vials and store at -20°C. Do not repeatedly freeze/thaw the recombinant protein standard as loss of activity may result.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles.

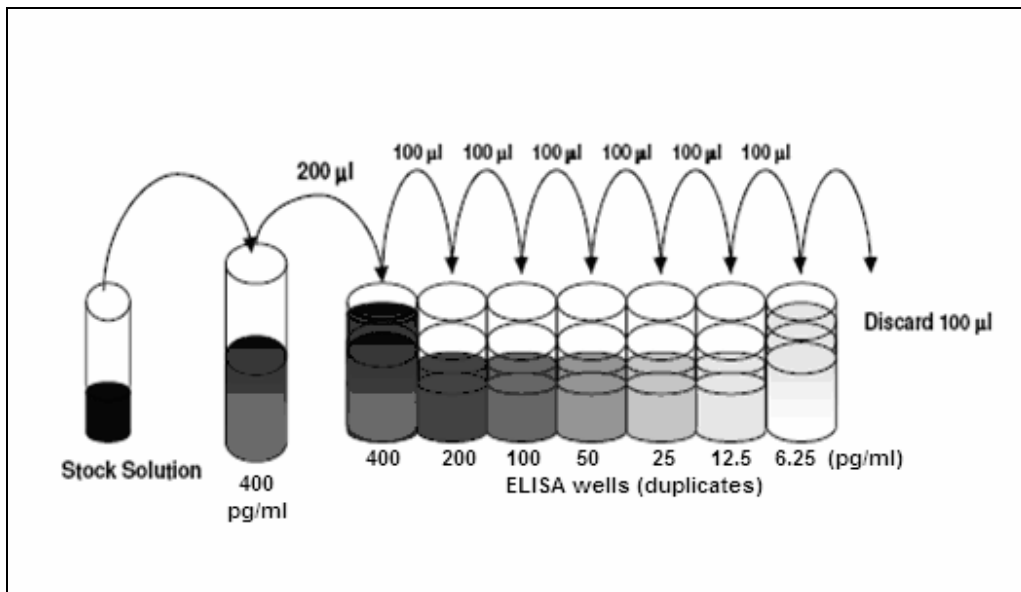
Reagent Preparation (all reagents should be diluted immediately prior to use):

1. 1X Wash Buffer
Dilution: To make 1X Wash Buffer, add 5ml of 20X Wash Buffer to 95ml of DI water. This is the working solution.
2. 1X Assay Diluent
Dilution: To make 1X Assay Diluent, add 1ml of 5X Assay Diluent to 4ml of DI water. This is the working solution.
3. Detection Antibody
Dilution 1:100, add 100 μ l of Detection Antibody to 9900 μ l of 1X Assay Diluent to make final volume to 10 ml.*
4. Avidin-HRP
Dilution 1:200, add 50 μ l of Detection Antibody to 9950 μ l of 1X Assay Diluent to make final volume to 10 ml.*
5. Upon first use, thaw 1 μ g tube of recombinant standard and quick-spin, aliquot into polypropylene vials, and store at -20°C. To run the assay, thaw and dilute the recombinant protein by adding 3.2 μ l of standard solution in 10ml of 1X Assay Diluent to prepare the top standard solution (320pg/ml).

*Note: It is recommended due to low volumes quick-spin down the sample for its settlement at the bottom of the vial provided, before dilution.

Assay Procedure:

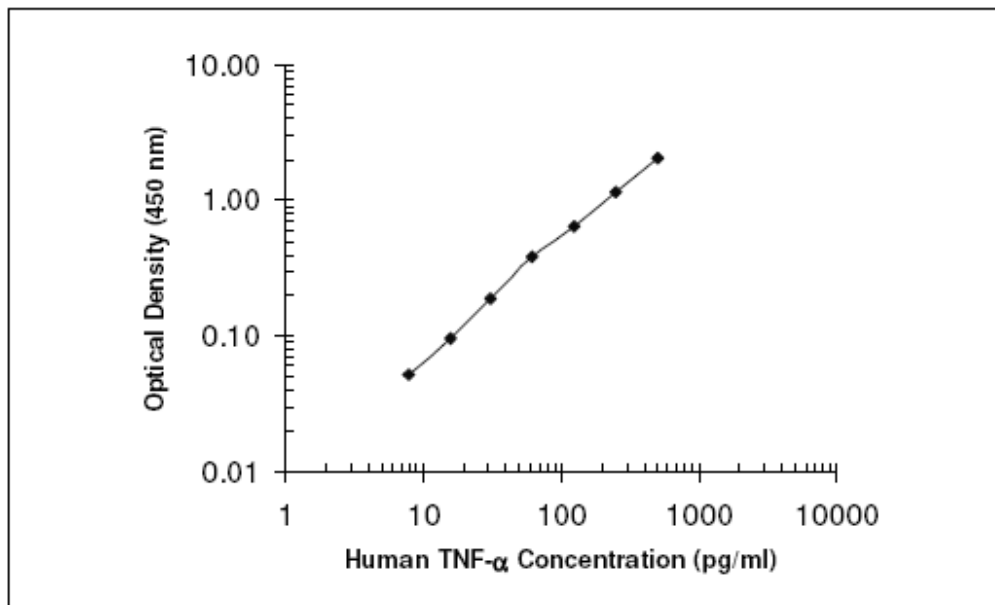
1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. Add 100 μ l/well of standards and samples to the plate. Perform six two-fold serial dilutions of the 400pg/ml top standard, either within the plate or in separate tubes. Thus, the human TNF- α standard concentrations are 400pg/ml, 200pg/ml, 100pg/ml, 50pg/ml, 25pg/ml, 12.5pg/ml, 6.25pg/ml. Assay Diluent serves as the zero standard (0pg/ml). Seal plate and incubate at 37°C for 2 hours.



3. Aspirate and wash plate 4 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
4. Add 100 μ l of diluted Detection Antibody solution to each well, seal plate and incubate at 37°C for 1 hour
5. Wash plate 4 times with Wash Buffer as in step 3.
6. Add 100 μ l of diluted Av-HRP solution to each well, seal plate and incubate at 37°C for 30 minutes.
7. Wash plate 4 times with Wash Buffer as in step 3.
8. Add 100 μ l of freshly mixed TMB Substrate solution and incubate in the dark for 45 minutes. Positive wells should turn a bluish color. It is not necessary to seal the plate during this step.
9. Stop reaction by adding 100 μ l of stop solution to each well. Positive wells should turn from blue to yellow.
10. Read absorbance at 450nm within 30 minutes of stopping reaction.

Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards, controls and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on log-log graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data:

This standard curve was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.

Sensitivity :

The minimum detectable dose of human TNF- α is typically less than 3pg/mL. The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. All samples measured less than the lowest TNF- α standard, 7.8pg/mL.

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Troubleshooting:

Problem	Possible cause	Investigation/Actions
High Absorbances	<ol style="list-style-type: none"> 1. Cross-contamination from other specimens 2. Insufficient or inefficient washing or reading 3. Wavelength of filter not correct. 4. High assay background. 5. Contaminated TMB 6. Incubation time too long or incubation temperature too high. 7. Incorrect dilution of serum 	<ul style="list-style-type: none"> > Repeat assay taking care when washing and pipetting. > Check washer efficiency > Check that the wavelength is 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650 nm. > Repeat assay and include a well that contains only sample diluent or sample absorbent (i.e. a blank well). > Check that TMB is colorless or faint blue. > Check incubation time and temperature. > Check incubator is at the correct temperature. > Repeat assay, ensuring correct serum dilution is used.
Low Absorbances	<ol style="list-style-type: none"> 1. Incubation time too short or incubation temperature too low. 2. Incorrect dilution or pipetting of sera 3. Incorrect filter wavelength. 4. Contaminated Conjugate solution. 5. Kit has expired. 6. Air blank reading high. 7. Incorrect storage of kit. 8. Kit reagents not equilibrated at room temperature 9. Incorrect reagents used. 10. Over washing of plate (e.g. inclusion of a long soak step). 	<ul style="list-style-type: none"> > Ensure time and temperature of assay incubation are correct. > Check incubator is set at the correct temperature. > Repeat assay ensuring correct dilutions and volumes are used. > Ensure controls are sufficiently mixed. > Check the wavelength is set at 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650nm. > Dispense conjugate directly from the bottle using clean pipette tip; avoid transferring Conjugate to another container if possible. > Do not return unused Conjugate to bottle. > Ensure all pipettes and probes used to dispense the Conjugates are clean and free from serum, detergent and bleach. > Check expiration date of kit and do not use if expired. Investigate causes of high background absorbance. > Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue/purple. > Allow sufficient time for reagents to equilibrate to room temperature prior to assay. > Check the reagents used match those listed on the specification sheet. > Repeat assay using recommended wash procedure.
Poor Duplicates	<ol style="list-style-type: none"> 1. Poor mixing of samples. 2. Poor pipette precision 3. Addition of reagents at inconstant timing intervals; reagent addition takes too long, air bubbles when adding reagents. 4. Inefficient washing - Wash buffer left in wells, inconsistent washing, inadequate washing. 5. Reader not calibrated or warmed up prior to plate reading. 6. Optical pathway not clean 7. Spillage of liquid from wells 8. Serum samples exhibit microbial growth, haemolysis or lipaemia. 9. Uneven well volumes due to evaporation. 	<ul style="list-style-type: none"> > Mix reagents gently and equilibrate to room temperature. > Calibration may need to be checked. > Check pipetting technique-change pipette tip for each sample and ensure excess liquid is wiped from the outside of the tip. > Use consistent timing when adding reagents. > Ensure all dilutions are made before commencing addition to plate. > Improve pipetting technique and skill. > Tap out wash buffer after washing. > Check wells are sufficiently and uniformly filled and aspirated when washing. > Check reader precision > Check reader manual to ascertain warm up time of instrument. > Gently wipe bottom of plate. > Check reader light source and detector are clean. > Repeat assay, taking care not to knock the plate or splash liquid > It is not recommended to use serum samples exhibiting microbial growth, haemolysis or lipaemia. > Cover plate with a lid or plate sealer (not provided).

All wells yellow	<ol style="list-style-type: none"> 1. Contaminated TMB. 2. Contaminated reagents (e.g. Conjugate, Wash buffer). 3. Incorrect dilution of serum. 4. Incorrect storage of kit. 5. Inefficient washing- Wash buffer left in wells, inconsistent washing, inadequate washing. 6. If Conjugate reconstitute is required – Conjugate reconstituted incorrectly. 	<ul style="list-style-type: none"> > Check TMB is colorless or faint blue. > Check reagents for turbidity. > Repeat assay, ensuring correct serum dilution is used. > Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue / purple. > Tap out wash buffer after washing. > Check wells are sufficiently and uniformly filled and aspirated when washing. > Repeat assay ensuring Conjugate is reconstituted according to assay method.
All wells negative	<ol style="list-style-type: none"> 1. Test not performed correctly – correct reagents not added or not added in the correct sequence. 2. Contaminated Conjugate solution. 3. Over- washing of plate (e.g. inclusion of a long soak step). 4. Incorrect storage of kit. 5. Wash Buffer made up with Stop Solution instead of Wash Buffer Concentrate 	<ul style="list-style-type: none"> > Check procedure and check for unused reagents. > Ensure that Stop Solution was not added before Conjugate or TMB. > Ensure that serum was diluted in correct Sample diluent; e.g. do not use Sample Absorbent for an IgG ELISA. > Dispense Conjugate directly from the bottle using a clean pipette tip; avoid transferring Conjugate to another container if possible. > Do not return unused Conjugate to bottle. > Ensure all pipettes and probes used to dispense the Conjugate are clean and free from serum, detergent and bleach. > Repeat assay using recommended wash procedure. > Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue / purple. > Ensure Wash Buffer is made up correctly.

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