abbexa®

# Chicken Tumor Necrosis Factor (TNF) ELISA Kit

**Catalog No: abx258416**

**Size:** 96T

**Range:** 7.8 pg/ml - 500 pg/ml

**Sensitivity:** < 3.10 pg/ml

**Storage:** Store the 96-well plate, Standards, and Detection Reagent(s) at -20°C, and the rest of the kit components at 4°C.

**Application:** The quantitative detection of TNF in Chicken serum, plasma, tissue homogenates, cell lysates, cell culture supernatant and other biological fluids.

**Principle of the Assay:** This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient TNF will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the TNF amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of TNF can be calculated.

**Kit Components**

- Pre-coated 96-Well Microplate: 12 x 8
- Standard: 2 tubes
- Wash Buffer: (30X) 20 ml
- Standard Diluent Buffer: 20 ml
- Detection Reagent A: (100X) 120 µl
- Detection Reagent B: (100X) 120 µl
- Diluent A: 12 ml
- Diluent B: 12 ml
- TMB Substrate: 9 ml
- Stop Solution: 6 ml
- Plate Sealer: 4

**Materials Required But Not Provided**

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Ice
- 0.01 mol/L PBS (pH 7.0 - 7.2)
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

For Reference Only



Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

# Protocol

### A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles. Keep samples on ice during preparation. Equivalate samples to room temperature prior to analysis.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 mins at 1000 × g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.
- **Tissue Homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 10000 × g for 5 mins and collect the supernatant.
- **Cell Lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS. Lyse the cells by ultra-sonification 4 times, or freeze at -20°C and thaw to room temperature 3 times. Centrifuge at 1500 × g for 10 mins at 2-8°C to remove cellular debris. Collect the supernatant.
- **Cell Culture Supernatant:** Centrifuge at approximately 1000 × g for 20 mins to remove precipitate.
- **Other Biological Fluids:** Centrifuge at approximately 1000 × g for 20 mins to remove precipitate. Analyse immediately or aliquot and store at -20°C or -80°C.

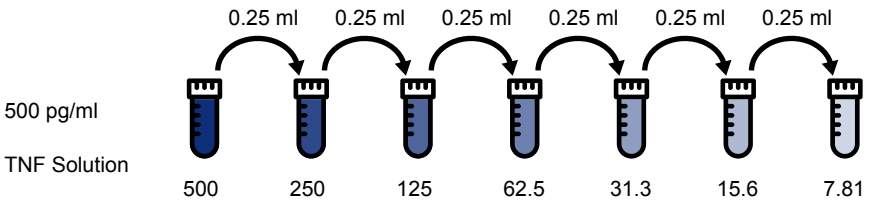
**Notes:**

- Samples must be diluted so that the expected concentration falls within the kit's range. The sample should be diluted in 0.01 mol/L PBS (PH=7.0-7.2).
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- $NaN_3$ cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

### B. Reagent Preparation

**Standard:** Prepare the Standard with 0.5 ml of Standard Diluent buffer to make the 500 pg/ml Standard Solution. This is the highest standard. Allow the reconstituted standard to sit for 10 mins, with gentle agitation prior to carrying out the serial dilutions. Avoid foaming or bubbles. Label tubes in preparation for the serial dilutions - *see the diagram below for reference*. Aliquot 0.25 ml of the Standard Diluent Buffer into each tube (apart from the highest standard tube). Add 0.25 ml of the highest standard solution into the 1st tube and mix thoroughly. Transfer 0.25 ml from the 1st to 2nd tube, mix thoroughly, and so on.

**Note:** Do not vortex the standard during reconstitution, as this will destabilize the protein. Once the standard has been reconstituted, it should be used within 15 mins. It is not recommended to reuse the reconstituted standard.




For Reference Only


Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

**Wash Buffer:** Dilute the concentrated Wash Buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

**Detection Reagent A Working Solution Preparation:** Prepare no more than 15 mins before the experiment.

1. Calculate the total volume of working solution required.
2. Dilute Detection Reagent A 100-fold with Diluent A, and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

**Detection Reagent B Working Solution Preparation:** Prepare no more than 15 mins before the experiment.

1. Calculate the total volume of working solution required.
2. Dilute Detection Reagent B 100-fold with Diluent B, and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

**C. Assay Protocol**

Prepare all standards, samples and reagents as directed above. Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate, and to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 100 µl of the diluted standards into the standard wells.
3. Aliquot 100 µl of Standard Diluent buffer into the control (zero) well.
4. Aliquot 100 µl of appropriately diluted sample into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
5. Cover the plate with a plate sealer and incubate for 1 hr at 37°C.
6. Remove the cover and discard the liquid. Do not wash.
7. Aliquot 100 µl of Detection Reagent A working solution to each well. Cover the plate with a plate sealer and incubate for 1 hr at 37°C.
8. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
9. Aliquot 100 µl of Detection Reagent B working solution to each well. Seal the plate and incubate for 30 mins at 37°C.
10. Remove the cover, discard the solution and repeat the wash process as described above, 5 times.
11. Aliquot 90 µl of TMB Substrate into each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 10-20 minutes. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
12. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

For calculation, average the OD 450 readings for each reference standard, and each sample, and then subtract the average control (zero) OD reading.

(Relative OD) = (OD of Each Well) – (OD of Zero Well)

The standard curve can be plotted as the relative OD of each reference standard solution (X), against the respective concentration of each standard solution (Y). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the concentrations from interpolation by the dilution factor, to obtain the concentration before dilution.

**Precautions:**

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.

For Reference Only

- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

**Technical Support**

For troubleshooting, frequently asked questions, and assistance, please visit:
https://www.abbexa.com/scientific-support/troubleshooting-and-faqs/elisa-kit-scientific-support or email us at support@abbexa.com.

**Precision:**

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of TNF were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of TNF were tested on 3 different plates, 8 replicates in each plate.

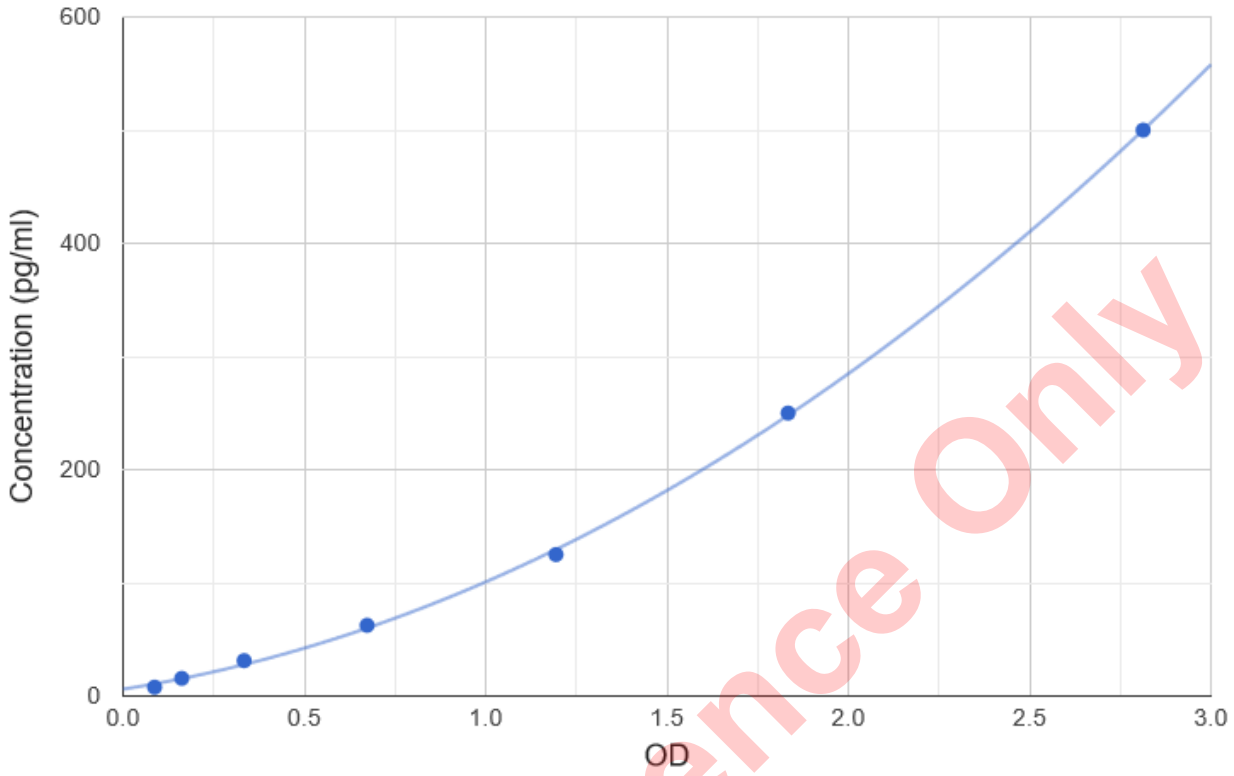
CV (%) = (Standard Deviation / Mean) × 100
Intra-Assay: CV < 10%
Inter-Assay: CV < 12%

**D. Typical Data and Standard Curve**

Typical Standard Curve Data is provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

For Reference Only


Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com




For Reference Only



Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com