Rat Tumor Necrosis Factor Alpha (TNFA) ELISA Development Kit

Catalogue No.:abx378099

Tumor Necrosis Factor Alpha (TNF-α) ELISA Development Kit for use in Sandwich ELISA assay development.

This ELISA Development Kit contains:

Component	5 × 96 tests	15 × 96 tests
Pretreated 96-well ELISA Plate	5	15
Capture Antibody	120 µl	350 µl
Biotin-Conjugated Detection Antibody	120 µl	350 µl
HRP-Conjugate	120 µl	350 µl
Standard (20000 pg)	1 vial	3 vials

Please note that quantities and concentrations may change between different batches.

It is recommended to use this ELISA Development Kit with abx471002 ELISA Development Support Kit (Sandwich Method); alternatively, the following solutions can be prepared separately:

- Coating Buffer: 1X Citrate-Buffered Saline
- Blocking Buffer: 1X PBS containing 0.5-3% BSA
- Wash Buffer: 3% Tris
- Standard and Sample Diluent Buffer: 1X PBS containing 0.5-3% BSA
- Antibody and HRP-Conjugate Diluent Buffer: 1X PBS containing 0.5-3% BSA
- Stop Solution: 5% Sulfuric Acid

Target: Tumor Necrosis Factor Alpha (TNFA)

Reactivity: Rat

Tested Applications: ELISA

Recommended dilutions: Capture Antibody: 1/500 - 1/1000, Biotin-conjugated Detection Antibody: 1/500 - 1/1000, HRP-Conjugate: 1/500 - 1/1000. Optimal dilutions/concentrations should be determined by the end user.

Reconstitution: Reconstitute the standard with 1 ml of Standard Diluent to obtain a stock standard solution of 20000 pg/ml. Further dilute by a factor of 10 to give the highest standard, 2000 pg/ml. Label tubes in preparation for the serial dilutions (1000, 500, 250, 125, 62.5, and 31.25 ng/ml). Aliquot 0.5 ml of the Standard Diluent into each tube. Add 0.5 ml of the highest standard solution into the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st to 2nd tube, mix thoroughly, and so on.

Storage: Aliquot and store at -20°C in the dark. Avoid repeated freeze/thaw cycles.

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

UniProt Primary AC:	P16599 (UniProt, ExPASy)
Test Range:	31.2 pg/ml - 2000 pg/ml
Detection Method:	Colormetric
Assay Type:	Sandwich
Sample Type:	Serum and plasma.
Sample Collection/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. • **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 as an anticoagulant. Centrifuge for 15 mins at 1000 × g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.
Note:	This product is for research use 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

Directions for use:

Bring all components to room temperature (18-25°C) and briefly spin or centrifuge the vials before use. Working solutions should be prepared and used immediately.

Recommended Procedure:

1. Dilute the Capture Antibody to working concentration using Coating Buffer. Immediately coat the 96-well plate with diluted Capture Antibody (100 µl per well). Seal the plate and incubate at 2-8 °C overnight.
2. Remove the liquid from each well. Do not wash. Block the plate with Blocking Buffer (200 µl per well) at 37 °C for 1 hour.
3. Remove the liquid from each well. Do not wash. Either proceed with the following steps immediately or dry the plate at 37 °C for 30 minutes, then store at -20 °C with dessicant for up to 6 months.
4. Add 100 µl of standards or sample into the appropriate wells. Cover with a plate sealer and incubate at 37 °C for 1.5 hours.
5. Remove the liquid from each well. Do not wash. Add appropriately diluted Biotin-Conjugated Detection Antibody (100 µl per well). Cover the plate with a new plate sealer and incubate at 37 °C for 1 hour.
6. Remove the liquid from each well. Wash with Wash Buffer (350 µl per well) and allow to soak for 1-2 min. Remove the liquid by inverting and tapping the plate on to absorbent paper. Repeat the wash process 3 times.
7. Add appropriately diluted HRP-Conjugate (100 µl per well). Cover the plate with a new plate sealer and incubate at 37 °C for 30 min.
8. Repeat the wash process in Step 6, for a total of 5 times.
9. Add Substrate Solution (90 µl per well). Cover the plate with a new plate sealer and incubate at 37 °C for 15-30 min. Keep the plate in the dark and avoid exposure to light.
10. Add Stop Solution (50 µl per well). Tap the side of the plate to ensure thorough mixing.
11. Measure the absorbance immediately using a microplate reader set at 450 nm.

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