

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Rat TNF Alpha ELISA Construction Kit

Product Number RRF425CK
Approx. 960 tests

Product Number **RRF425CKC**
With Developing Reagents:

Capture Antibody	100.0 ug	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	0.5mL
Antigen Standard	1.0 or 5.0 ug	TMB Substrate (50 mL x 2)	
		WASH Buffer (20X)	100 mL

DESCRIPTION:

This ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard sufficient for **approximately** ten microplates.

Working concentrations must be optimized by customer.

Note: Reconstitute components only when ready to run assay.

CAPTURE ANTIBODY:

Provided as lyophilized, **100.0 ug**, additive-free. Reconstitute in 0.50 mL sterile water (200.0 ug/mL). (FREEZE aliquots for long-term storage)

TRACER ANTIBODY:

Provided as 25 ug (0.5 mL Liquid or lyophilized - **see vial**) of Biotin labeled, antigen-affinity purified antibody, additive-free. For lyophilized : Reconstitute in 500 uL sterile water **containing 0.1% BSA**. (FREEZE aliquots for long-term storage).

For vial provided as **Liquid - Store refrigerated only - contains preservative**

STANDARD: * Provided as 1.0 ug or 5.0 ug (**see vial**) of recombinant Rat TNF Alpha - see vial. Quick-spin and reconstitute in 100 uL of sterile water. Further dilutions can be made in PBS.

DEVELOPING REAGENTS: Supplied with catalog # ending in "CKC".

- ELISA Coating/ Blocking Reagent (EA150C) 50.0 mL (5X Solution)
- Streptavidin-HRP (S100180C) 0.5 mL - store @ -20 Deg. C.
- TMB Substrate Solutions - Part A and Part B (50.0 mL each)
cat # ES200C
- WASH Buffer (20X) Dilute 1 part with 19 parts distilled water.

- **HANDLING/ STORAGE:** Reconstitute reagents when ready to build ELISA assay. Antibodies: Capture can be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months. Standard (rec. Rat TNF Alpha) can be stored in liquid state (@ 4 Deg. C.) For up to one week, or store **frozen, with addition of 0.1% BSA**, at -20 Deg. C. for up to 3 months. AVOID repeat freeze-thaw.

Biotin tracer antibody provided as Liquid (0.5 mL) should be stored refrigerated only.

MATERIALS RECOMMENDED:

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404
 Tween -20.
 BSA
 Streptavidin-HRP: ANTIGENIX Cat no. S100180 or similar
 TMB Substrate
 Dubelco's PBS (10X)
 ANTIGENIX **ELISA Coating Stabilizer** (cat no: **EA150**)

RECOMMENDED SOLUTIONS:

See ANTIGENIX Developing reagents above.

PBS: Dilute to 1XPBS in sterile water
 WASH BUFFER: ANTIGENIX **WB200** or 0.05% Tween-20 in PBS.
 BLOCK BUFFER: **use ANTIGENIX AMERICA coating stabilizer (EA150)**
or 1% BSA in PBS
 Substrate Solution: TMB Substrate Solution
 Diluent: 0.05% Tween-20, 0.1% BSA in PBS
 2N Sulfuric acid (stop solution).

PLATE PREPARATION:

1. Dilute **portion** of capture antibody with 0.05M Carbonate buffer (or PBS) to concentration 1.0 ug/mL.

Immediately add 100 uL to each ELISA well. Seal the plate and incubate overnight at room temperature.

2. Aspirate wells to remove all liquid and wash **4 times** using 300 uL of wash buffer per well. After last wash, add 200 uL ANTIGENIX AMERICA **ELISA coating stabilizer - recommended! -(cat # EA150)** and incubate for 60 minutes at room temperature. (With coating stabilizer, DO NOT let plate dry prior to use of coating stabilizer. This will **stabilize and Block in one step!** Refer to data sheet EA150 for complete description of use.
3. With ANTIGENIX coating stabilizer (**recommended**) aspirate plate but **DO NOT WASH**. For extended storage- dry plate in humidity controlled chamber or similar. (see data sheet EA150). With standard block reagent, aspirate plate and wash 3X with 300 uL wash buffer.

PROTOCOL:

STANDARD/SAMPLE: Dilute **a portion of the** standard (store unused standard in aliquots, high concentration, frozen -20 Deg. C.) from **4.0 ng/mL** to zero in diluent (serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. Incubate at room temp. for approx. 2 hours.

DETECTION: Aspirate and wash plate 4 times. **Dilute** detection (Biotin Tracer) antibody in diluent to concentration of **0.20 ug/mL**. Add 100 uL per well. **Incubate** at room temperature for **1-2 hours**. Note: detection antibody can be used in approximate range of 0.10 - 0.50 ug/mL, you may need to optimize for subsequent plates.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. **1:2,000** in diluent (follow recommended dilution of manufacturer). (May need to optimize) Add 100 uL per well, incubate 30 minutes at room temperature.

SUBSTRATE: Aspirate and wash plate 4 times. Add 100 uL substrate solution to each well. Incubate at room temp. for color development. Monitor color development with plate reader at 650 nm wavelength. (for blue color). Stop the color reaction after 10 - 20 minutes by adding 100 uL of 2N Sulfuric acid to each well. Then, **read plate @ 450 nm**. After addition of stop solution.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.30 units for the zero standard concentration, or 2.0 units for the highest standard concentration. Monitor the plate every 5 minutes for approximately 30 minutes.

X-Reactivity Data:

X-Reactivity observed @ 40-50 ng/mL with following factors:

Mouse TNF Alpha

Minimal (1%) X-reactivity observed with following:

Human TNF Alpha

No measurable X-reactivity observed with following factors:

Rat: GM-CSF; GRO-beta; GRO/KC; CNTF; IL-1 alpha and beta;
IL-13; IFN gamma; RANTES; MCP-1; SCF

Mouse: JE

Human: TNF beta; sTNF R1 and R2; MCP-1