

# ANTIGENIX AMERICA, INC.

## PRODUCT INFORMATION SUMMARY

### Rat TNF Alpha ELISA Construction Kit

Product Number RRF425CK  
Approx. 960 tests

Product Number **RRF425CKC**  
**With Developing Reagents:**

<b>Capture Antibody</b>	<b>100.0 ug</b>	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