

# ANTIGENIX AMERICA, INC.

## PRODUCT INFORMATION SUMMARY

### Rat RANTES ELISA Construction Kit

Product Number RRF424CK  
Approx. 960 tests

Product Number **RRF424CKC**  
**With Developing Reagents:**

<b>Capture Antibody</b>	<b>50.0 ug</b>	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25. or 50.0 ug	Streptavidin-HRP	0.5 mL
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, **50.0 ug ( see vial)** , additive-free. Reconstitute in 0.50 mL sterile water (200.0 ug/mL or 100.0 ug/mL).

#### TRACER ANTIBODY:

Provided as 25 ug (lyophilized) **or 0.5 mL** liquid @ 100 ug/mL - **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 RANTES. Quick-spin and reconstitute in 50 uL of sterile water. Further dilutions can be made in 0.05% Tween-20, 0.1% BSA in PBS.

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

- ELISA Coating/ Blocking Reagent ( EA150C) 50.0 mL ( 5X Solution)
- Streptavidin-HRP ( S100180C) 1.0 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 and lyophilized Tracer) 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 RANTES) can be stored in liquid state ( @ 4 Deg. C.) For up to one week, or store **frozen, add 0.1% BSA**, at -20 Deg. C. for up to 2 months. AVOID repeat freeze-thaw.

For Biotin tracer antibody provided as 0.5 mL liquid - STORE refrigerated only - contains preservative.

**MATERIALS RECOMMENDED:**

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404  
Tween -20.  
BSA  
Streptavidin-HRP: ANTIGENIX Cat no. **S100180** or similar  
TMB Substrate (ANTIGENIX cat # **ES200**)  
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 (ANTIGENIX # ES200)  
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 **0.5 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**. 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 **2.0 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** portion of detection (Biotin Tracer) antibody in diluent to concentration of **0.25 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. ( follow directions from manufacturer) 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 within 30 minutes of addition of stop solution.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.25 units for the zero standard concentration, or 2.0 units for the highest standard concentration.

**X-REACTIVITY DATA:** X-Reactive with Mouse RANTES

**No measurable** X-Reactivity @ 40-50 ng/mL was observed with following:

Rat MIP-1 alpha and beta; Rat MCP-1

Human: RANTES, MEC, TECK

Mouse: MIP-1 alpha and beta; C-10; MCP-2, MCP-3, MCP-5; MEC;  
Eotaxin, JE; MIP-2, MIP-3

**RESEARCH USE ONLY -NOT For DIAGNOSTIC USE**

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