

## PRODUCT INFORMATION SUMMARY

### Human RANTES ELISA Construction Kit

Product Number RHF520CK  
Approx. 960 tests

Product Number **RHF520CKC**  
**With Developing Reagents:**

Capture Antibody	100.0 ug	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 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

**RHF520CKP** - with Developing Reagents and 10 plates

#### 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 ug, additive-free. Reconstitute in 0.50 mL sterile water (200.0 ug/mL).

#### TRACER ANTIBODY:

Provided as 25 ug of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute in 500 uL sterile water **containing 0.1% BSA**.

**STANDARD:** Provided as 1.0 ug or 5.0 ug of recombinant Human RANTES.

Quick-spin and reconstitute in 50 uL of sterile water. Further dilutions can be made in 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) 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 and 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. Human RANTES) 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 2 months. AVOID repeat freeze-thaw.

#### **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: 0.1% 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 **2.0 ng/mL** to zero in diluent (8 point, 1:2 serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. **Incubate** at room temp. for approx. 90 minutes

**DETECTION:** Aspirate and wash plate 4 times. **Dilute** portion of detection (Biotin Tracer) antibody in diluent to concentration of **0.20 ug/mL**. Add 100 uL per well. **Incubate** at room temperature for **approx. 30 minutes -1 hour**. 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. **Stop** the color reaction after **10 - 20 minutes** by adding 100 uL of 2N Sulfuric acid to each well. Then, **read plate at 450 nm** after 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.

NOTE: Kit can be ordered with developing reagents ( cat v# vends in 'CKC')- see page 1.

**X-REACTIVITY DATA:**

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

Rat: RANTES

Mouse: RANTES; MCP-2; MCP-3, MIP-1 alpha

Human: MCP-2, MCP-3, MCP-4, MIP-1 alpha and beta; Eotaxin;  
LEC.

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