PRODUCT INFORMATION SUMMARY

Mouse Rantes ELISA Construction Kit

Product Number RMF432CK Approx. 960 tests Product Number RMF432CKC With Developing Reagents:

Capture Antibody50.0 ugBiotin tracer25.0 or 50.0ugAntigen Standard1.0 or 5.0 ug

ELISA Coating Stabilizer 50 mL Streptavidin-HRP 0.5mL TMB Substrate (50 mL x 2) WASH Buffer (20X) 100 mL

DESCRIPTION:

Mouse RANTES is a CC-chemokine that has chemotactic effects upon monocytes, eosinophils, basophils, and memory T cells. The abilily of RANTES to inhibit HIV and SIV has been reported. It signals via the CCR group of receptors. Mouse Rantes is a 68 aa. prtoein with molecular weight of 7.8 kDA. 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 ug, additive-free. Reconstitute in 0.50 mL sterile water (100.0 ug/mL).

TRACER ANTIBODY:

Provided as 25 ug (lyohilized) or 0.5 mL liquid (50.0 ug)- **see** vial- of Biotin labeled, antigen-affinity purified antibody, additive-free. For 25.0 ug lyophilized - reconstitute in 500 uL sterile water **containing 0.1% BSA**. Liquid vial (0.5 mL) contains 50.0 ug.

STANDARD: Provided as 1.0 ug or 5.0 ug (see vial) of recombinant Mouse 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 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. Mouse 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.

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 blank (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 0.25 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 0.20 - 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). The color reaction may be stopped 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 0.25 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.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

X-Reactivity Data:

Following factors were observed to exhibit **100%** X-reactivity @ 40-50 ng/mL:

Rat RANTES

Following factors were observed to exhibit **no** measurable X-reactivity @ 40-50 ng/mL:

Human: RANTES, MEC
Rat: MIP-1 alpha and beta; MCP-1
Mouse: Eotaxin; MIP-1 alpha, beta, and gamma; MIP-2, MIP-3;
 MEC; C-10; JE (MCP-1); MCP-2, MCP-3

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