

REV: 9/13

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Rat MCP-1 ELISA Construction Kit

Product Number RRF423CK
Approx. 960 tests

Product Number **RRF423CKC**
With Developing Reagents:

Capture Antibody	50.0 ug	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	0.5mL
Antigen Standard	1.0 or 2.0 ug	TMB Substrate (50 mL x 2)	
		WASH Buffer (20X)	100 mL

DESCRIPTION:

Rat MCP-1 ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard for development of **approximately** ten microplate assays. Working concentrations must be optimized by customer.

Note: Reconstitute components only when ready to run assay.

CAPTURE ANTIBODY:

Provided as lyophilized, **50.0 ug**, additive-free. Reconstitute in 0.50 mL sterile water (100.0 ug/mL). (FREEZE aliquots for long-term storage)

TRACER ANTIBODY:

Provided as 25 ug (lyophilized) **or as 0.5 mL liquid @ 50.0 ug/mL (see vial)** of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute the 25 ug (lyophilized vial) in 500 uL sterile water **containing 0.1% BSA.** (FREEZE aliquots for long-term storage). -

****For liquid vial store refrigerated only (contains preservative)**.**

STANDARD: Provided as 1.0 ug or 2.0 ug (see vial) of recombinant Rat MCP-1. Quick-spin and reconstitute in 50 uL of sterile water (pH 7.2). 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) 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.

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

Standard (rec. Rat MCP-1) 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** or similar
 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 (cat # **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**. 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 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 **0.20 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- (use PBS with 0.1% BSA) (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 for substrate preparation and handling from manufacturer's data sheet). Incubate at room temp. for color development. Monitor color development with plate reader at 650 nm wavelength. (for blue color). **Add 100 uL stop solution** (2N Sulfuric acid) to stop color reaction after **10 - 20 minutes**. 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 1.6 units for the highest standard concentration.

X-reactivity data:

X-Reactivity (100%) observed @ 40-50 ng/mL with following factor: Mouse MCP-1 (JE)

Minimal (1%) X-Reactivity observed with following:
Mouse MCP-5

No X-reactivity observed with following factors:
Mouse MCP-3, MIP-1 alpha
Rat: Mip1- alpha and beta; RANTES; MIP-2/GRO Beta; GRO/KC
Human: MCP-1; MCP-2, MCP-3, MCP-4; MIP-1 alpha; IL-8; GRO;
GRO Gamma; CXCL16

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

NOTE: Kit can be ordered with developing reagents:

(cat# ends in '**CKC**'). - see page 1.

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