

REV: 1/24

ANTIGENIX AMERICA Inc

PRODUCT INFORMATION SUMMARY

Human MIP-3 Beta ELISA Construction Kit

Product Number RHF610CK
Approx. 960 tests

Product Number **RHF610CKC**
With Developing Reagents:

| | | | |
|-----------------------|----------|----------------------------|-------|
| Capture Antibody | 100.0 ug | ELISA Coating Stabilizer | 50 mL |
| Biotin-Labeled tracer | 25.0 ug | Streptavidin-HRP | 0.5mL |
| Antigen Standard | 5.0 ug | TMB Substrate (50 mL x 2) | |
| | | Wash Buffer (20X) | |

RHF610CKP: with Developing Reagents & ten plates

DESCRIPTION:

Human MIP-3 Beta ELISA CONSTRUCTION Kit provides antigen affinity purified polyclonal capture and tracer antibodies, and antigen standard for development of **approximately** five to ten microplate assays. 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 distilled or sterile water

TRACER ANTIBODY:

Provided as 25 ug (lyophilized or as 0.5 mL) of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute in 500 uL distilled water **containing 0.1% BSA.** (Store aliquots @ -20 Deg. C. for long-term storage)

STANDARD: Provided as 5.0 ug of recombinant Human MIP-3 Beta. Quick-spin and reconstitute in 50 uL of distilled water (pH 7.2). 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 @ -20 Degrees C. for up to 6 months. Standard (rec. MIP-3 Beta) 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
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: 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: 0.1% BSA in PBS
2N Sulfuric acid (stop solution).

PLATE PREPARATION:

1. Dilute **100 uL 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. 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, @ -20 Deg. C.) from 1.0 ng/mL (1:2, 8-point serial dilution) to zero blank in diluent.

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. 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 for substrate preparation and handling from manufacturer's data sheet). **Incubate** at room temp. for color development. **Stop** the color reaction after 10 - 15 minutes by adding 100 uL of 2N Sulfuric acid (stop solution) 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.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

NOTE: Kit can be ordered with the developing reagents and ten (10) blank ELISA plates as RHF610CKP

ANTIGENIX AMERICA Inc.

WWW.ANTIGENIX.COM