

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

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