

RE: 2/14

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human BMP-2 ELISA Construction Kit

Product Number RHF913CK
Approx. 960 tests

Product Number RHF913CKC
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	1.0 ug or 2.0 ug	TMB Substrate	(50 mL x 2)
		WASH Buffer (20X)	100 mL

DESCRIPTION:

Human BMP-2 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, 100.0 ug, additive-free. Reconstitute in 0.50 mL sterile water (200.0 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 lyophilized vial in 500 uL sterile water **containing 0.1% BSA**. (FREEZE aliquots for long-term storage)

For biotin tracer provided as 0.5 mL liquid- **STORE** refrigerated only.

STANDARD: Provided as 1.0 ug or 2.0 ug (see vial) of recombinant Human BMP-2. Quick-spin and reconstitute in 50 uL of sterile water **containing BSA (50 ug BSA per 1 ug protein)** (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.

Biotin tracer provided as 0.5 mL liquid- STORE refrigerated only.

Standard (rec. BMP-2) 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 **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 (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**. (Approx. **1:250** from 50.0 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 (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 (blue color) with plate reader at 650 nm wavelength. . **Stop** the color reaction after **10 - 20 minutes** by adding 100 uL of 2N Sulfuric acid to each well. **Read plate** at **450 nm** (correction @ 650 nm), after reaction is stopped.

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.

X-REACTIVITY DATA:

Minimal (10%) X reactivity was observed @ 40-50 ng/mL with following factors: Human BMP-4

No X-reactivity was observed with following factors:

Human: BMP-3; BMP-6; BMP-7; BMP-13; CTGF; Activin A; WNT-1;
CTGF^L; Follistatin; Noggin; TGF beta 1 and 2; Myostatin;
GDF-3

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE