

REV. 6/15

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human Osteopontin "SUPER X" ELISA Kit

Product Number **RHF860CKX**

5 Plate Kit (5 x 96 tests)

RHF860CKX2 (2 x 96 tests)

RHF860CKX1 (96 tests)

Items Provided:

PRE-COATED ELISA Plates (Single, 2 or 5 plates)
Biotin-Labeled tracer 0.5 mL or 25.0 ug (see vial)
Antigen Standard 10.0 ug
Streptavidin-HRP 0.5 mL
TMB Substrate 25 mL x 2 (15 mL - 2 plate)
Wash Buffer Concentrate 125 mL (20X) (50 mL - 2 plate)

DESCRIPTION:

Human **Osteopontin SUPER X ELISA Kit** provides **PRE-COATED** microplates (coated with antigen affinity purified capture antibody stabilized with our proprietary ELISA coating/blocking reagent. A biotin labeled tracer antibody, antigen standard, HRP developing reagents and wash buffer are included.

Reactivity with various sample types, including serum/plasma samples, is evaluated by customer.

Note: Reconstitute components only when ready to run assay.

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 10.0 ug (**lyophilized**) of recombinant Human Osteopontin. Quick-spin and **reconstitute in 100 uL distilled water** (pH 8.0) - concentration approx. 100.0 ug/mL. (DO NOT VORTEX) Further dilutions can be made in Diluent (ANTIGENIX ED100) or 0.1% BSA in PBS.

DEVELOPING REAGENTS:

- Streptavidin-HRP (S100180CX) 0.5 mL - **store @ -20 Deg. C.**
- TMB Substrate Solutions - Part A and Part B (30.0 mL each)
cat # ES200CX
- Wash Buffer Concentrate (20X concentrate) Mix 1 volume of wash buffer with 19 volumes of distilled water. Stable for one month @ 4 Deg. C. once mixed to working volume.
- Diluent (ED100) 10X- Mix 1 part with 9 parts distilled water

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. Biotin Tracer Antibody provided as 25.0 ug lyophilized 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 antibody provided as 0.5 mL liquid - STORE REFRIGERATED - contains preservative.

Standard (rec. Osteopontin) should be stored @ -20 Deg. C. - **with addition of 0.1% BSA**, for up to 2 months. AVOID repeat freeze-thaw.

Pre-coated **ELISA plates** should be stored refrigerated (@ 4 Deg.C.) in sealed plastic bag with desiccant pack, stable until expiration date on kit box.

Store Streptavidin-HRP (S100180CX) @ -20 Deg. C.

Store TMB solutions and wash buffer concentrate at 4 Deg. C.
CAUTION: Substrate Solution B contains 20% acetone. FLAMMABLE.
Keep away from sources of heat or flame.

MATERIALS RECOMMENDED:

Tween -20.
BSA (ELISA grade only, protease-free)
Dubelco's PBS (10X)
PBS: Dilute to 1XPBS in sterile water
Diluent: use : ANTIGENIX ED100 or 0.1% BSA in PBS
2N Sulfuric acid - Stop Solution.

PLATE PREPARATION:

Plates are **pre-coated** with capture antibody and blocked/stabilized with ANTIGENIX proprietary ELISA coating stabilizer (EA150) and are **ready to use!**

Store plates refrigerated (@ 4 Deg. C.) in sealed plastic bags with desiccant pack. -until exp. date on kit box.

PROTOCOL:

STANDARD/SAMPLE: Dilute **a portion of the** standard (store unused standard in aliquots, high concentration, frozen -20 Deg. C. with addition of 0.1% BSA) **from 2.0 ng/mL** to zero in diluent (8 point - 1:2 serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. **Incubate** at room temp. for **60 minutes**.

DETECTION: Aspirate and wash plate 4 times. Dilute 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 **40 minutes**.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. **Dilute Streptavidin-HRP** conjugate approx. **1:2,500** in diluent. (May need to optimize) Add 100 uL per well, incubate **30 minutes** at room temperature.

SUBSTRATE: Prepare substrate solution no more than 15 minutes before last incubation of assay: Mix one part TMB Solution A with one part TMB Solution B in a clean container. If, upon mixing, TMB solution turns blue - TMB solution is **contaminated- DO NOT USE**. Use mixed substrate solution **WITHIN 2 Hours, and AVOID DIRECT LIGHT**.

Aspirate and wash plate 4 times. **Note:** Wash steps are critical! **Add 100 uL substrate** solution to each well. Incubate at room temp. for color development. **Add 100 uL of Stop solution** (2N Sulfuric Acid) **after approx. 10 minutes** to stop color development - gently tap plate to mix. **Read plate at 450 nm** within 30 minutes of addition of stop solution.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.30 units for the zero standard concentration, or 2.0 units for the highest standard concentration.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

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