**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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