REV. 03/11

PRODUCT INFORMATION SUMMARY

Human EMAP-II "SUPER X" ELISA Kit

Product Number **RHF902CKX**
5 Plate Kit (5 x 96 tests)
RHF902CKX2 (2 plate kit)
RHF902CKX1 (Single plate)

Items Provided:

PRE-COATED ELISA Plates (Single, 2 or 5 plates)

Biotin-Labeled tracer	25.0 ug
Antigen Standard	5.0 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 Endothelial -Monocyte Activating Polypeptide (EMAP-II) **SUPER X ELISA Kit** provides one, two or five **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 of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute in 500 uL sterile water **containing 0.1% BSA**. (FREEZE aliquots for long-term storage)

STANDARD: Provided as 5.0 ug of recombinant Human EMAP-II. Quick-spin and **reconstitute in distilled water** (pH 7.4) - concentration approx. 100 ug/mL. Further dilutions can be made in 0.05% Tween-20, 0.1% BSA in PBS.

DEVELOPING REAGENTS:

- Streptavidin-HRP (S100180CX) 1.0 mL - **store** @ **-20 Deg. C**.
- TMB Substrate Solutions - Part A and Part B (25.0 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.

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. Tracer Antibody be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months. Standard (rec. EMAP-II) 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. Pre-coated ELISA plates should be stored in sealed plastic bag with desiccant pack, and are stable for one year from date of receipt. If needed, additional components (antigen standard or developing reagents) can be purchased as separate items to run additional stored plates - inquire.

Store Streptavidin-HRP (S100180XH) **frozen** @ -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 : 0.05% Tween-20, 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. Plates can be stored for one year from data of receipt.

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 **40.0 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 **2 hours**.

DETECTION: Aspirate and wash plate 4 times. **Dilute** detection (Biotin Tracer) antibody in diluent to concentration of **0.25 ug/mL**. Add 100 uL per well. **Incubate** at room temperature for **60 minutes**.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. Dilute Streptavidin-HRP conjugate approx. **1:700** 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. You may monitor color development with plate reader at 650 nm wavelength. (for blue color). Add 100 uL of Stop solution, 2N Sulfuric Acid,**after 10-15 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 0.35 units for the zero standard concentration, or 2.0 units for the highest standard concentration.

WARRANTY:

Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE