ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human Exodus-2
"SUPER X" ELISA Kit

Product Number RHF806CKX

5 Plate Kit (5 x 96 tests)

RHF806CKX2 (2 Plate Kit)

RHF806CKX1 (single plate Kit)

Items Provided:

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

Biotin-Labeled tracer 25.0 ug or 0.5 mL (see vial)
Antigen Standard 1.0 ug or 5.0 ug (see vial)

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 Exodus-2 (CCL21) **SUPER X ELISA Kit** provides single, 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 (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 1.0 or 5.0 ug (see vial) of recombinant Exodus-2 (CCL21). Quick-spin and reconstitute in distilled water (pH 8.0) - concentration approx. 100.0 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- provided as lyophilized- after
reconstitution- 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. Exodus-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. Precoated ELISA plates should be stored refrigerated (@ 4 Deg.C.) in sealed plastic bag with desiccant pack, and are stable for one year from date of receipt.

Store Streptavidin-HRP (S100180CX) 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 until expiration date on kit.

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 4.0 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 90 minutes.

DETECTION: Aspirate and wash plate 4 times. **Dilute** detection (Biotin Tracer) antibody in diluent to concentration of **0.20 ug/mL**. Add 100 uL per well. **Incubate** at room temperature for **1 hour**.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. Dilute Streptavidin-HRP conjugate approx. 1:2,000 in diluent. 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 ofstop solution (2N Sulfuric Acid) after approx. 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 0.25 units for the zero standard concentration, or 1.6 units for the highest standard concentration.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

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