REV: 3/17

ANTIGENIX AMERICA Inc.

PRODUCT INFORMATION SUMMARY

Human sCD22 "SUPER X" ELISA Kit

Product Number **RHF225CKX**

5 Plate Kit (5 x 96 tests)
RHF225CKX2 (2 x 96 tests)
RHF225CKX1 (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)

ELISA Diluent (10X); STOP Solution (1X)

DESCRIPTION:

Human sol. CD22 **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.

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 5.0 ug of recombinant Human sCD22. Quick-spin and **reconstitute in 50.0 - 100.0 uL uL distilled water** - concentration approx. 100.0 ug/mL. Further dilutions can be made in ELISA diluent provided 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 (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.
- ELISA diluent (ED100) (10X) -Dilute 1 part with 9 parts distilled water
- STOP Solution (1X) 2N sulfuric acid- **CAUTION: Caustic**

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. CD22) 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.

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
STOP Solution: Use ANTIGENIX STP100 or 2N Sulfuric acid

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 box.

PROTOCOL:
MODIFIED PROCEDURE: (Add Standard/Sample and Biotin together)

PREPARE 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 blank in diluent (make 8-point, 1:2 serial dilution).

PREPARE Biotin DETECTION: Dilute portion of the detection (Biotin Tracer) antibody in diluent to concentration of **0.25 ug/mL.** (Dilute approx. 1:200 from 50.0 ug/mL)

Add 100 ul of each **Standard** (or sample) **and** 50.0 uL of the **Biotin Detection** antibody **together** to each well in duplicate.

Incubate at Room Temp. for **90 minutes.** Aspirate and WASH plate 4 Times.

STREPTAVIDIN-HRP: **Dilute** Streptavidin-HRP conjugate approx. **1:2,000** in diluent. **Add** 100 uL per well, **incubate** for **1 hour** 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. **10-15 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 0.30 units for the zero standard concentration, or 2.2 units for the highest standard concentration.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE