

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**

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