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. Quickspin 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

ANTIGENIX AMERICA Inc. WWW.ANTIGENIX.com