ANTIGENIX AMERICA Inc.

PRODUCT INFORMATION SUMMARY

Human IL-2 "SUPER X" ELISA Kit

Product Number **RH200020CKX**
5 Plate Kit (5 x 96 tests)
RH200020CKX2 (2 x 96 tests)
RH200020CKX1 (96 tests)

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** 10.0 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)
Diluent (10X)

DESCRIPTION:

Human Interleukin 2 (IL-2) **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 0.5 mL liquid (see vial) of Biotin labeled, antigen-affinity purified antibody, additive-free. For 25.0 ug lyophilized : Reconstitute in 200 uL sterile water **containing 0.1% BSA.** (FREEZE aliquots for long-term storage)

For liquid vial (0.5 mL) - store refrigerated only.

STANDARD: Provided as 1.0 ug **or** 10.0 ug (see vial) of recombinant Human IL-2. Quick-spin and **reconstitute in distilled water** (pH 8.0) - concentration approx. 100.0 ug/mL. Further dilutions can be made in Diluent provided.

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. Concentrate may be stored at room temp.

- Diluent (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. Reconstituted Tracer Antibody be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months.

For tracer antibody (0.5 mL) provided liquid- strore refrigerated only- contains preservative.

Standard (rec. IL-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. Pre-coated ELISA plates should be stored refrigerated (@ 4 Deg.C.) in sealed plastic bag with desiccant pack, and are stable until expiration date on kit box.

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 ANTIGENIX ED100 or 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.

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 **3.0 ng/mL** to zero in diluent (1:2 , 8 point -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 **0.2 ug/mL.** (approx. 1:250 from 50.0 ug/mL) Add 100 uL per well. **Incubate** at room temperature for **40 minutes.**

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. **1:2,000** 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. **Add** 100 uL of **Stop solution** (2N Sulfuric Acid) **after approx. 10 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