REV 3/13

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human IL-17 "SUPER X" ELISA Kit

Product Number **RH170172CKX**

5 Plate Kit (5 x 96 tests)
RH170172CKX2 (2 x 96 tests)
RH170172CKX1 **(** 96 tests**)**

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 (15.0 mL - 2 plate)
Wash Buffer Concentrate	125 mL (20X) (50 mL - 2 plate)

DESCRIPTION:

Human Interleukin 17 (IL-17A) **SUPER X ELISA Kit** provides 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 of Biotin labeled, antigen-affinity purified antibody, additive-free. Reconstitute in 500 uL sterile water **containing 0.1% BSA**. (FREEZE aliquots for long-term storage)

STANDARD: Provided as 1.0 ug or 5.0 ug (**see vial)** of recombinant Human IL-17A. Quick-spin and **reconstitute in distilled water** (pH 8.0) - concentration approx. 0.1 mg/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 be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months. Standard (rec. IL-17A) 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 for one year from date of receipt.

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 : 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 for one year from data of receipt.

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 **4.0 ng/mL** to zero in diluent (serial dilution).

PREPARE Biotin DETECTION: Dilute portion of the detection (Biotin Tracer) antibody in diluent to concentration of **0.20 ug/mL**.

Add 100 ul of each Standard (or sample) **and** 50.0 uL of the Biotin Detection antibody 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. **1:1,000** in diluent. (May need to optimize) 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 **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 2.0 units for the highest standard concentration.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE