REV. 5/11

PRODUCT INFORMATION SUMMARY

Rat IP-10 "SUPER X" ELISA Kit

Product Number **RRF314CKX**
5 Plate Kit (5 x 96 tests)
RRF314CKX2 (2 Plate Kit)
RRF314CKX1 (Single Plate Kit)

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	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)

DESCRIPTION:

Rat IP-10 **SUPER X ELISA Kit** provides one, 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.

Final working concentrations and assay range may need to be optimized by customer. 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 as 0.5 mL liquid @ 33.0 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 Rat IP-10. Quick-spin and **reconstitute in distilled water** (pH 8.0) - concentration approx. **100.0 ug/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 (25 ug lyophilized) be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months.

Biotin Tracer antibody provided liquid (0.5 mL) - **Store Refrigerated only -** contains preservative.

Standard (rec. Rat IP-10) 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 (S100180CX) **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 **2.0 ng/mL** to zero in diluent (serial dilution).

PREPARE Biotin DETECTION: Dilute portion of the detection (Biotin Tracer) antibody in diluent to concentration of **0.10 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 **1 hour.** Aspirate and WASH plate 4 Times.

STREPTAVIDIN-HRP: **Dilute** Streptavidin-HRP conjugate approx. **1:3,000** in diluent. (May need to optimize) Add 100 uL per well, incubate for **45 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. Monitor color development with plate reader at 650 nm wavelength. (for blue color). **Add** 100 uL of **Stop solution** (2N Sulfuric Acid) **within 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 2.0 units for the highest standard concentration.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE