PRODUCT INFORMATION SUMMARY

Human IL-9 "SUPER X" ELISA Kit

Product Number **RH900020CKX**

5 Plate Kit (5 x 96 tests)
RH900020CKX2 (2 x 96 tests)
RH900020CKX1 (1 x 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	2.0 ug or 1.0 ug (see vial)
Streptavidin-HRP	0.5 mL
TMB Substrate	25 mL x 2 (15 mL x 2 - 2 plate format)
Wash Buffer Concentrate	125 mL (20X) (50 mL - 2 plate)

DESCRIPTION:

Human IL-9 **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.

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 1.0 ug or 2.0 ug (see vial) of recombinant Human IL-9. 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) 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) - Dilute 1 part with 19 parts distilled water

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. Biotin Tracer Antibody provided as 25.0 ug lyophilized can 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 as 0.5mL liquid - STORE refrigerated - contains preservative.

Standard (rec. IL-9) 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 desicant 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)
Dubelco's PBS (10X)
PBS: Dilute to 1XPBS in sterile water
Diluent: use : 0.05% Tween-20, 0.1% BSA in PBS

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:

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 (serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. Incubate at room temp. for **1 hour**.

DETECTION: Aspirate and wash plate 4 times. Dilute detection (Biotin Tracer) antibody in diluent to concentration of **0.20 ug/mL**. Add 100 uL per well. Incubate at room temperature for **1 hour**.

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) 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 0.25 units for the zero standard concentration, or 1.6 units for the highest standard concentration. Monitor the plate every 5 minutes for approximately 30 minutes.

X-Reactivity Data:

The following factors were tested @ 40-50 ng/mL:

Minimal (1%) X reactivity was observed with: Human: IL-2; IL-6

No X-Reactivity was observed with:

Mouse IL-9

Human: IL-12; IL-15, IL-2Ra; IL-6Ra.