

ANTIGENIX AMERICA, INC.

REV: 6/11-2

PRODUCT INFORMATION SUMMARY

Human sRANKL

Product Number **RHF740CKX**

"SUPER X" ELISA Kit

5 Plate Kit (5 x 96 tests)

Product Number **RHF740CKX2** (2 x 96 tests)

RHF740CKX1 (single plate)

Items Provided:

PRE-COATED ELISA Plates (1,2, or 5 plates)
Biotin-Labeled tracer 25.0 ug or 0.5 mL (**see vial**)
Antigen Standard 1.0 ug or 2.0 ug
Streptavidin-HRP 0.5 mL
TMB Substrate 25 mL x 2 (15.0 mL or 10 mL)
Wash Buffer Concentrate 125 mL (20X); or 50 mL or 25 mL

DESCRIPTION:

Human sRANKL **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 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 1.0 ug or 2.0 ug (**see vial**) of recombinant Human sRANKL. 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 or

- 15.0 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. Store refrigerated.

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 0.5 mL liquid -STORE refrigerated only - contains preservative.

Standard (rec. sRANKL) 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, and are stable until expiration date on kit..

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 : 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 until expiration date on kit box.

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 **2.0 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 **0.10 ug/mL**. Add 100 uL per well. **Incubate** at room temperature

for **30 minutes**.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. **1:2,000** in diluent (PBS with 0.1% BSA). (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 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.

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

X-REACTIVITY DATA:

Minimal (1%) X-Reactivity was observed with the following factors @ 40-50 ng/mL:

Human: TWEAK; TNF alpha; sRANKR

No measurable X-reactivity was observed with the following:

Human: sTRAIL; IL-20; sCD40L; Eotaxin-3; TNF Beta; sFasL; 4-1BBL

Rat and Mouse: sRANKL; TNF alpha

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