

REV: 8/11

PRODUCT INFORMATION SUMMARY

Human TWEAK "SUPER X" ELISA Kit

Product Number **RHF770CKX**
5 Plate Kit (5 x 96 tests)
RHF770CKX2 (2 Plate Kit)
RHF770CKX1 (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 5.0 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)

DESCRIPTION:

Human TWEAK **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 @ 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 5.0 ug (see vial) of recombinant Human TWEAK. Quick-spin and **reconstitute in 100 uL of 10mM sodium phosphate** (pH 7.5) - concentration approx. 50.0 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. Concentrate may be stored at room temp.

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. Tracer Antibody provided as 25.0 ug lyophilized- after reconstitution - 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. TWEAK) 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 3 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:

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 **0.20 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. **1:2,500** 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 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.

X-Reactivity Data:

The following factors were observed to react with **no** measurable X-reactivity:

Human: sTRAIL; sTRAILR1 and R2; TWEAK-R; BAFF; CD40L; TNF alpha and beta; sRANKL; sRANKR; OPG; sFasL; 4-1BBL

Mouse: sRANKL; TNF alpha; sCD40L

Rat TNF alpha.