

REV: 1/13

ANTIGENIX AMERICA, INC.

PRODUCT INFORMATION SUMMARY

Human sRANKL ELISA Construction Kit

Product Number RHF740CK
Approx. 960 tests

Product Number **RHF740CKC**
With Developing Reagents:

Capture Antibody 100.0 or 300.0ug
Biotin-Labeled tracer **see vial**
Antigen Standard 1.0 or 2.0 ug

ELISA Coating Stabilizer 50 mL
Streptavidin-HRP 0.5mL
TMB Substrate (50 mL x 2)
WASH Buffer (20X) 100 mL

DESCRIPTION:

Human sRANKL. This ELISA CONSTRUCTION Kit provides antigen affinity purified capture and tracer antibodies, and antigen standard sufficient for **approximately** ten microplates. Working concentrations must be optimized by customer.

Note: Reconstitute components only when ready to run assay.

CAPTURE ANTIBODY:

Provided as lyophilized, **100.0 ug or 300.0 ug (see vial)**, additive-free. Reconstitute in 0.5 mL sterile water (200.0 ug/mL - 600.0 mg/mL). (FREEZE aliquots for long-term storage)

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 50 uL of sterile water. Further dilutions can be made in 0.05% Tween-20, 0.1% BSA in PBS.

DEVELOPING REAGENTS: Supplied with catalog # ending in "CKC".

- ELISA Coating/ Blocking Reagent (EA150C) 50.0 mL (5X Solution)
- Streptavidin-HRP (S100180C) 1.0 mL - store @ -20 Deg. C.
- TMB Substrate Solutions - Part A and Part B (50.0 mL each) cat # ES200C
- WASH Buffer (20X)-Dilute 1 part with 19 parts distilled water

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. Antibodies provided as lyophilized - after reconstitution- (Capture and Tracer) 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 provided as 0.5 mL liquid- STORE refrigerated only.

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.

MATERIALS RECOMMENDED:

ELISA Microplates: Nunc Maxisorp, Prod. # 4420404

Tween -20.

BSA

Streptavidin-HRP: ANTIGENIX Cat no. **S100180** or similar

TMB Substrate, ANTIGENIX cat No: **ES200**

Dubelco's PBS (10X)

ANTIGENIX **ELISA Coating Stabilizer** (cat no: **EA150**)

RECOMMENDED SOLUTIONS:

See ANTIGENIX Developing Reagents above

PBS: Dilute to 1XPBS in sterile water

WASH BUFFER: ANTIGENIX WB200 or 0.05% Tween-20 in PBS.

BLOCK BUFFER: **use ANTIGENIX AMERICA coating stabilizer (EA150) or 1% BSA in PBS**

Substrate Solution: TMB Substrate Solution

Diluent: 0.05% Tween-20, 0.1% BSA in PBS

2N Sulfuric acid (stop solution).

PLATE PREPARATION:

1. Dilute **portion** of capture antibody with 0.05M Carbonate buffer (or PBS) to concentration **1.0 ug/mL - 2.0 ug/mL.**

Immediately add 100 uL to each ELISA well. Seal the plate and incubate overnight at room temperature.

2. Aspirate wells to remove all liquid and wash **4 times** using 300 uL of wash buffer per well. After last wash, add 200 uL ANTIGENIX AMERICA **ELISA coating stabilizer - recommended - (cat # EA150)** and incubate for 60 minutes at room temperature. (With coating stabilizer, DO NOT let plate dry prior to use of coating stabilizer. This will **stabilize and Block in one step!** Refer to data sheet EA150 for complete description of use.

With ANTIGENIX coating stabilizer (**recommended**) aspirate plate but **DO NOT WASH.** For extended storage- dry plate in humidity controlled chamber or similar. (see data sheet EA150). With standard block reagent, aspirate plate and wash 3X with 300 uL wash

buffer.

PROTOCOL:

STANDARD/SAMPLE: Dilute **a portion of the** standard (store unused standard in aliquots, high concentration, frozen -20 Deg. C.) 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 approx. 2 hours.

DETECTION: Aspirate and wash plate 4 times. **Dilute** a portion of detection (Biotin Tracer) antibody in diluent to concentration of **0.20 ug/mL**. Add 100 uL per well. Incubate at room temperature for 1-2 hours. May need to adjust/optimize in range of 0.10 - 0.50 ug/mL.

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. **1:2,000** in diluent (follow recommended dilution of manufacturer). (May need to optimize) Add 100 uL per well, incubate 30 minutes at room temperature.

SUBSTRATE: Aspirate and wash plate 4 times. 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). Stop the color reaction after 10 - 20 minutes by adding 100 uL of 2N Sulfuric acid to each well. Then, **read plate @ 450 nm** after 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.

NOTE: Kit can be ordered with developing reagents (cat# ends in 'CKC') - see page 1.

X-Reactivity Data:

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

Mouse and Rat sRANKL

No X-Reactivity was observed with following:

Human: OPG; sCD40L; sRANKR; sTRAIL; TWEAK; TNF alpha; FasL

Mouse and Rat TNF alpha

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

ANTIGENIX AMERICA Inc.

WWW.ANTIGENIX.COM