

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**

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