

REV: 7/11

## PRODUCT INFORMATION SUMMARY

### Human sol.CD40 Ligand ELISA Construction Kit

Product Number RHF901CK  
Approx. 960 tests

Product Number **RHF901CKC**  
**With Developing Reagents:**

<b>Capture Antibody</b>	<b>100.0</b> ug	ELISA Coating Stabilizer	50 mL
Biotin-Labeled tracer	25.0 ug	Streptavidin-HRP	1.0mL
Antigen Standard	1.0ug or 10.0 ug	TMB Substrate ( 50 mL x 2)	
		WASH Buffer (20X)	100 mL

#### DESCRIPTION:

Human Soluble CD40 Ligand is also known as TRAP molecule, contains the receptor binding site for CD40L, a protein involved in helper function of T cells. Human sCD40 Ligand is a 149 aa. protein with molecular weight of 16.3 kDA. 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**, additive-free. Reconstitute in 0.50 mL sterile water (200.0 ug/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 10.0 ug ( see vial) of recombinant Human sCD40 Ligand. Quick-spin and **reconstitute in** 0.5 mL sterile or distilled 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. Reconstituted Antibodies (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 antibody provided as 0.5 mL liquid - STORE refrigerated only- contains preservative.

Standard ( rec. SCD40 Ligand) can be stored in liquid state ( @ 4 Deg. C.) For up to one week, or store **frozen** at -20 Deg. C. with **addition of 0.1% BSA**, 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  
Dubelco's PBS (10X)  
ANTIGENIX **ELISA Coating Stabilizer** ( cat no: **EA150**)

**RECOMMENDED SOLUTIONS:**

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, ANTIGENIX **ES200**  
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.

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 (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.
3. With ANTIGENIX coating stabilizer ( **recommended** ) aspirate plate but **DO NOT WASH**. 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 detection (Biotin Tracer) antibody in diluent to concentration of 0.25 ug/mL. Add 100 uL per well. Incubate at room temperature for 1-2 hours. Note: detection antibody can be used in approximate range of 0.10 - 0.50 ug/mL, you may need to optimize for subsequent plates.

**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). **Add 100 uL stop solution** ( 2N sulfuric acid), **after 10-20 minutes** to stop color reaction. **Read plate @ 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 2.0 units for the highest standard concentration.

**X-REACTIVITY DATA: tested @ 40-50 ng/mL**

Minimal X-reactivity was observed with following factors:

Mouse CD40L

No measurable X-reactivity observed with following:

Human: TWEAK; BAFF; FasR; FasL, sRANKL; sRANKR; sTRAIL; sTRAIL-RI and RII; 4-1BBL; IL-1 alpha; TNF alpha and beta

Rat TNF alpha

Mouse: TNF alpha; sRANKL

**RESEARCH USE ONLY -NOT For DIAGNOSTIC USE**

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