

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:

Capture Antibody	100.0 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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