HUMAN CD40 LIGAND ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CD40 LIGAND CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, EDTA PLASMA

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN CD40 LIGAND ELISA
Catalog No.	SK00242-01
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µl
Sample Type	Cell Culture Supernates, Serum, Plasma
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human CD40 Ligand
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2°C - 8°C

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INTRODUCTION

Human CD40 Ligand immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human CD40 Ligand in cell culture supernates, serum, and EDTA plasma. It contains recombinant human CD40 Ligand and antibodies raised against this protein. It has been shown to accurately quantify recombinant human CD40 Ligand. Results obtained with naturally occurring CD40Lsamples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human CD40 Ligand.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CD40 Ligand has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD40 Ligand present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for CD40 Ligand is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of CD40 Ligand bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_The kit should not be used beyond the expiration date on the kit label.

_Do not mix or substitute reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
CD40LMicroplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against human CD40 Ligand.	**242-01-01**	**1 plate**
CD40 Ligand Standard – 1000 pg/vial of recombinant human CD40 Ligand in a buffered protein base with preservatives; lyophilized.	**242-01-02**	**2 vials**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of Biotinylated antibody against CD40 Ligand with preservatives; lyophilized.	**242-01-03**	**1 vial**
Positive Control - one vial of recombinant human CD40 Ligand, lyophilized	**242-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 75 uL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Wash Buffer - 50mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted standard and detection antibody concentrate could be stored for up to a month at -70°C. Diluted standard working solution and positive control should be prepared and used immediately.

Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation.

SAMPLE PREPARATION

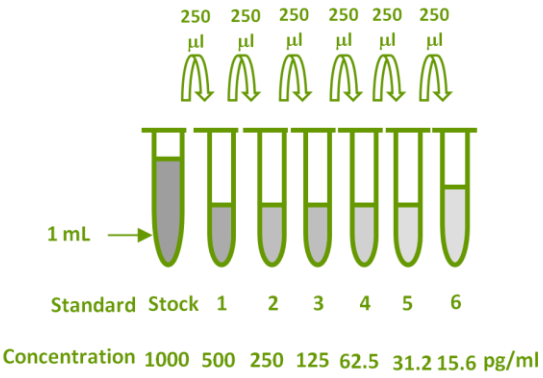
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

CD40LStandard - Refer to vial label for reconstitution volume. Reconstitute the **CD40 Ligand Standard** with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	500 µl	1000 pg/ml
# 1	250 µl of stock	250 µl	500 pg/ml
# 2	250 µl of 1	250 µl	250 pg/ml
# 3	250 µl of 2	250 µl	125 pg/ml
# 4	250 µl of 3	250 µl	62.5 pg/ml
# 5	250 µl of 4	250 µl	31.25 pg/ml
# 6	250 µl of 5	250 µl	15.6 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1X

working solution of Streptavidin- HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

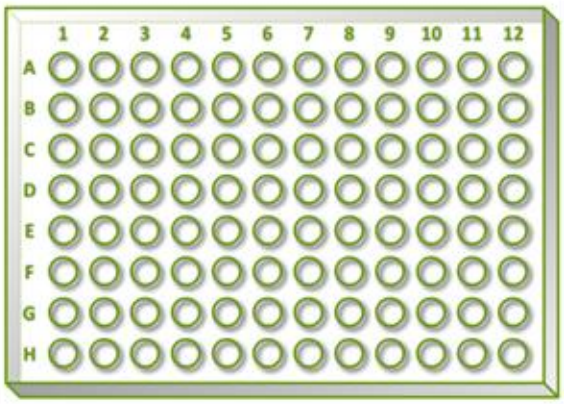
Bring all reagents and samples to room temperature before use. It is recommended that standards and positive control be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank well (A2, A3).
4. Add 100 µL of **Standard** (from B2, B3 to G2, G3 and G4, G5), **sample, or positive control** (F4, F5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1X Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 20-30 minutes at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log- log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the CD40L concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human CD40 Ligand.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of CD40L was 5pg/mL.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.071)
15.6	0.029
31.25	0.045
62.5	0.093
125	0.174
250	0.361
500	0.701
1000	1.527
4000	2.884

- Lot No.:
- Positive Control:

SPECIFICITY

PROTEIN	CROSSREACTIVITY (%)
Human CD40 Ligand	100
Mouse CD40 Ligand	0
Human sCD40	0
Human sCD36	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 20-30 min on the bench top. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min