Human sCD40L ELISA KIT

Catalog Number	Size
EA100113	48 Tests

Human sCD40L ELISA KIT

For the quantitative determination of human CD40 Ligand (CD40L) concentrations in cell culture supernates, serum, and plasma. This package insert must be read in its entirety before using this product. If you have questions or experience problems with this product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

CD40 Ligand (CD40L), also known as CD154, gp39, TNFSF5, TRAP (TNF-Related Activation Protein) or TBAM (T-cell B-cell Activating Molecule), is a multifunctional ligand in the TNF superfamily (1-4). Interaction between CD40 and CD40L is critical to the control of thymus-dependent humoral immunity and cell-mediated immune responses (5-10). The major component of the contact-dependent signal leading to B cell activation is CD40L. CD40L stimulates B cell secretion of immunoglobulin isotypes in the presence of cytokines.

CD40L is a 39 kDa, 261 amino acid (aa) glycoprotein that can form homotrimers typical of other TNFSF members (1-4, 11, 12). Proteolytic cleavage can also produce 15-18 kDa soluble forms of CD40L (13, 14). Activated T cells and platelets express both a membrane-associated and a soluble form of CD40L (sCD40L) (13, 15, 16). Platelet activation during plasma and serum sample preparation can result in artificially elevated sCD40L levels (17-20). Conversely, serum samples stored above 2-8 °C show a progressive loss of the sCD40L signal (21). sCD40L lacks the transmembrane region and a portion of the extracellular domain but contains the entire TNF- homology region. Both the membrane-bound and soluble forms of CD40L are active (22).

The receptor for CD40L is CD40, a member of the TNF receptor superfamily (TNFRSF5). Interaction of CD40L with CD40 not only induces proliferation and isotype switching in B lymphocytes but also mediates a broad variety of other immune and inflammatory responses (5-7). CD40 signaling has been linked with pathogenic processes of chronic inflammatory diseases such as autoimmune diseases, neurodegenerative disorders, graft-versus-host disease, cancer, and atherosclerosis (8). The loss of interaction between CD40 and CD40L can result in impairment of T lymphocyte function, B lymphocyte differentiation, and monocyte function.

CD40L is expressed primarily on activated CD4+ T cells; however, vascular endothelial cells, smooth muscle cells, macrophages, basophils, eosinophils, monocytes, dendritic cells, fibroblasts, and mast cells also express CD40L. Cytokine stimulation (e.g. IL-1β, TNF-α, or IFN-γ) can increase surface levels and de novo synthesis of CD40L in certain cell types (23). Hyper-IgM syndrome (HIGM) is an immunodeficiency characterized by elevated concentrations of serum IgM and the absence of serum IgG, IgA, and IgE. It is caused by mutations within the CD40L gene leading to defective expression on the membrane of activated T lymphocytes (24, 25). B lymphocytes from HIGM patients express functional CD40 and respond

normally to wild-type CD40L, but their T lymphocytes are unable to stimulate CD40 signaling pathways (26, 27).

CD40L may play multiple roles in HIV infection (28). It may contribute to viral replication control by inducing HIV-suppressive chemokines, by downregulating monocyte cell surface expression of CCR5 and CD4, and by supporting the production of anti-HIV antibodies and cytotoxic T cells (28-31). It can also promote HIV replication in CD4+ T lymphocytes by activating antigen-presenting cells, subsequently leading to increased CD4+ T cell activation (28). With the onset of AIDS, CD40L-expressing CD4+ T cells become selectively depleted. This loss may explain the similarity between the opportunistic infections characteristic of AIDS and those observed with congenital CD40L deficiency (28).

Elevated levels of sCD40L have been observed in sera from patients with systemic lupus erythematosus (SLE), chronic lymphocytic leukemia (CLL), and unstable angina (32-34). A direct relationship has been seen between disease severity and sCD40L in SLE patient sera (32). Aberrant expression of CD40L may thus contribute to autoantibody secretion in SLE through activation of bystander B lymphocytes, including cells that have been exposed to self antigens (32). Prolonged survival of malignant CLL cells may be linked to elevated levels of biologically active sCD40L (33). CD40L can mediate the resistance of CLL cells to apoptosis by Fas Ligand and fludarabine (33). Enhanced levels of both soluble and membrane-bound forms of CD40L in angina patients suggests that the CD40L-CD40 interaction may play a pathogenic role in the atherosclerotic process and in promoting acute coronary syndromes (34).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sCD40L has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sCD40L present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for sCD40L is added to the wells and binds to the combination of capture antibody- sCD40L in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of sCD40L present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven sCD40L standard dilutions and sCD40L sample concentration determined.

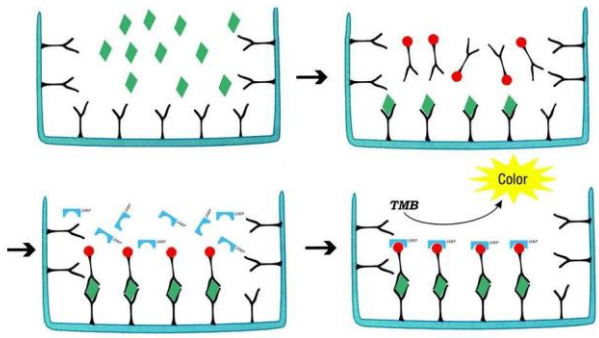

Figure 1:Schematic diagram of the assay

REAGENTS

1. Aluminium pouches with a Microwell Plate coated with antibody to human sCD40L (8X12)
2. 2 vials human sCD40L Standard lyophilized, 10000pg/ml upon reconstitution
3. 2 vials HRP-Conjugate solution
4. 1 bottle Standard /sample Diluent
5. 1 bottle Streptavidin-HRP Diluent
6. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
7. 1 vial Substrate Solution
8. 1 vial Stop Solution
9. 4 pieces Adhesive Films
10. Package insert

NOTE: [96 Tests]

STORAGE

Table 1: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 – 8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="6">May be stored for up to 1 month at 2 – 8℃.**</td></tr>
<tr><td>HRP-Conjugate solution</td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20℃.
Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 – 8℃.**</td></tr>
</table>

**Provided this is within the expiration date of the kit

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000μL.
3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃ and 8℃. After use all reagents should be immediately returned to cold storage (2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.

5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).
   Note: The normal human serum or plasma samples are suggested

to make a 1:5 dilution.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 1mL of Standard /sample Diluent. This reconstitution produces a stock solution of 10000 pg /ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 500µl of Standard/sample Diluent into the 5000 pg/ml tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 10000 pg/ml standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/ml).

   If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

4. Working solution of HRP-Conjugate: Make a 1:100 dilution of the concentrated HRP-Conjugate solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

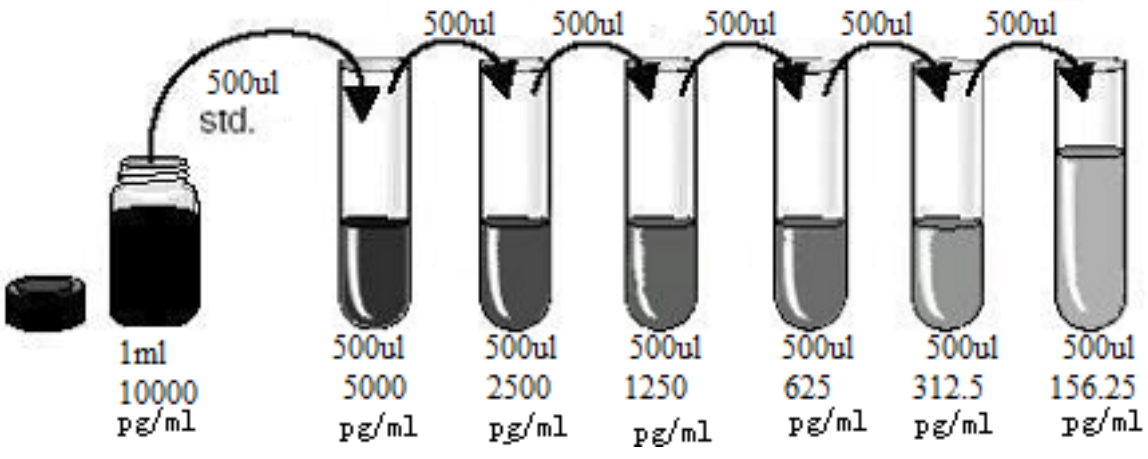

Figure 2: Preparation of sCD40L standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100µl of Standard, control, or sample, per well, then add 100µl of the working solution of HRP-Conjugate to each well. Cover with the adhesive strip provided and incubate 2 hours at RT. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350 µl) using a squirt bottle, manifold dispenser or auto-washer. 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.
5. Add 100µl of Substrate Solution to each well. Incubate for 20-30 minutes at RT. Avoid placing the plate in direct light.
6. Add 100µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (Optionally 650nm as the reference wave length; 610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

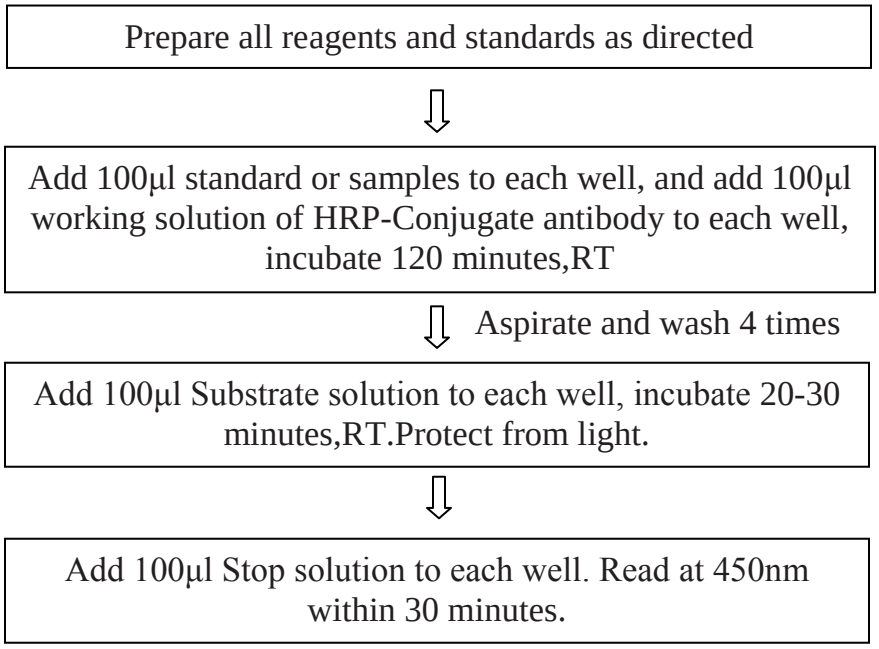

Figure 3: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate

diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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.
3. The data may be linearized by plotting the log of the sCD40L 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.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 2:Typical data using the sCD40L ELISA (Measuring wavelength: 450nm, Reference wavelength: 650nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.011	0.009	0.010	——
156.25	0.076	0.071	0.074	0.064
312.5	0.139	0.132	0.136	0.126
625	0.255	0.249	0.252	0.242
1250	0.471	0.465	0.468	0.458
2500	0.918	0.909	0.914	0.904
5000	1.829	1.814	1.822	1.812
10000	3.477	3.459	3.468	3.458

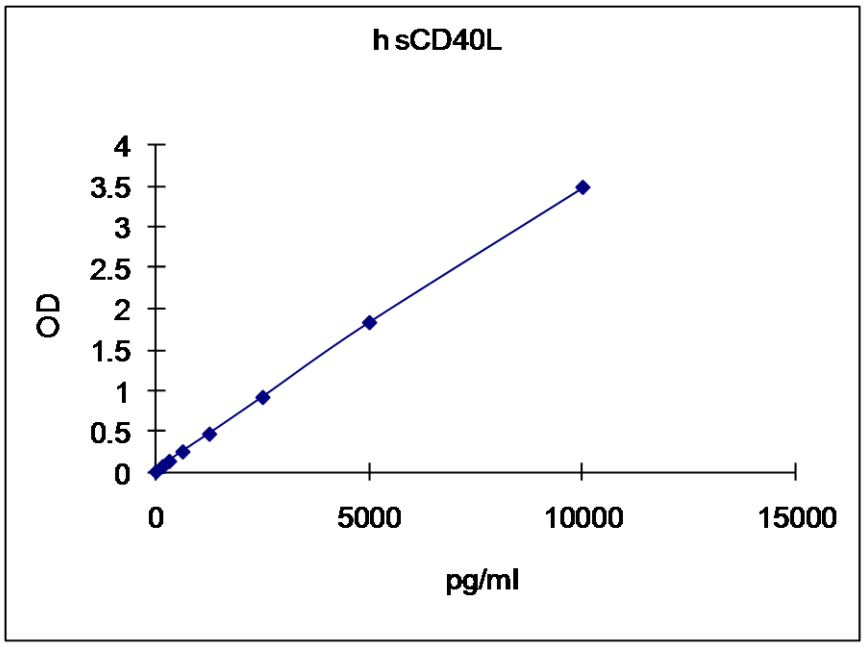

Figure 4:Representative standard curve for sCD40L ELISA. sCD40L was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

SENSITIVITY, SPECIFICITY AND REPEATABILITY

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 40 pg/mL.
3. **SPECIFICITY:** This assay recognizes both natural and recombinant human sCD40L. The factors listed below were prepared at 50 ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 3: Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant rat
CD4	CD40L	IFN-γ
CD40	Fas	IL-1α
Fas	CT-1	IL-1β
IFN-γ	IFN-γ	IL-2
IL-1α	IL-1α	IL-4
IL-1β	IL-1β	IL-6
IL-2	IL-2	IL-10
IL-4	IL-4	TNF-α

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If you have any questions, please tell us!