Human sCD40L ELISA Kit

Cat：RK00053

This ELISA kit used for quantitation of human Cd40 Ligand (sCD40L) concentration in cell culture supernate, serum and plasma. For research use only, and it's highly recommended to read throughly of this manual before using the product.

Manufactured by

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Contents

Introduction

CD40 belongs to the TNF receptor superfamily. While the biological role of some of the ligand-receptor pairs in this family still remains obscure, CD40 has proven its importance. A key role of CD40/CD40ligand interactions in immune activation, particularly in T-cell dependent B cell responses is anticipated. This molecule as well as the other ligands of the family share the property of co-stimulation of T-cell proliferation and are all expressed by activated T-cells. The programmed cell death has been suggested to be involved in clonal elimination of self-reactive lymphocytes for the normal function of the immune system. Interaction with membrane bound self antigens may eliminate self-reactive nature B cells by apoptosis. Antigen-receptor mediated B cell apoptosis is blocked when a signal is transduced via the CD40 molecule on the B cell surface.

Because the ligand of CD40 (CD40L) is expressed on activated T helper cells, B cells may escape from apoptosis and are activated when the immune system interacts with foreign antigens, which are normally able to activate T-helper cells. Thus the CD40/CD40L interaction plays a central role in the various phases of the B cell response to T-dependent antigens. Taken together, B cells can participate in regulating their own destruction. Protection against Fas-dependent apoptosis afforded by immunoglobulin-receptor engagement may constitute a fail-safe mechanism that eliminates bystander B cells activated by CD40L-expressing T cells, but ensures survival of antigen-specific B cells. BMS239CE and BMS239TENCE human sCD40L 5 CD40 Ligand is expressed on the surface of activated CD4+ T cells, basophils, and mast cells. Binding of CD40L to its receptor, CD40, on the surface of B cells stimulates B-cell proliferation, adhesion and differentiation. A soluble isoform of CD40L has been shown to exist in the circulation. This soluble molecule is a homotrimer of a 18kDa protein exhibiting

full activity in B cell proliferation and differentiation assays, is able to rescue B cells from apoptosis and binds soluble CD40. CD40L is discussed in relation to a potential role in supporting B cell tumors and it has been discovered that the molecular defect in the X-linked Hyper-IgM-Syndrome is targeted to the CD40L gene, it is functional involved in B cell hybridomas and chronic lymphocytic leukemia as well as several autoimmune diseases.

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 colored product TMB 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. A standard curve is prepared from seven sCD40L standard dilutions and sCD40L sample concentration determined.

Materials Provided

Description	Size (192T)	Size (96T)	Size (48T)	Storage	Cat NO.
Human sCD40L antibody coated plate	(8×12) ×2	8×12	8×6	4°C	RM00240
Human sCD40L Standard lyophilized	4 vials	2 vials	1 vial	4°C	RM00237
Standard/sample Diluent (R1)	2 bottles ×20 mL	1 bottle ×20 mL	1 bottle ×20 mL	4°C	RM00023
Human sCD40L concentrated biotin conjugate antibody (100×)	2 vials ×120 µL	1vial ×120 µL	1 vial ×60 µL	4°C	RM00238
Biotin-Conjugate antibody Diluent (R2)	1 bottle ×32 mL	1 bottle × 16 mL	1 bottle × 16 mL	4°C	RM00024
Streptavidin-HRP concentrated (100×)	2 vials ×120 µL	1 vial ×120 µL	1 vial ×60 µL	4°C	RM00239

Streptavidin-HRP Diluent(R3)	1 bottle ×32 mL	1 bottle ×16 mL	1 bottle ×16 mL	4°C	RM00025
Wash Buffer (20x)	2 bottles ×30 mL	1 bottle × 30 mL	1 bottle ×30 mL	4°C	RM00026
Substrate Solution (Dark)	2 bottles ×12 mL	1 bottle ×12 mL	1 bottle ×6 mL	4°C	RM00027
Stop Solution	1 bottle ×24 mL	1 bottle ×12 mL	1 bottle ×12 mL	4°C	RM00028
Plate Sealers	8 strips	4 strips	2 strips		
Specification	1				

Sample Collection And Storage

1. **Cell Culture Supernates:**
   Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.
2. **Serum:**
   Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.
3. **Plasma**
   Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot

and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles.

4. **Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.**
5. **Dilution:**

   Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

Precautions For Use

1. **Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.**
2. **Stop Solution contains strong acid. Wear eye, hand, and face protection.**
3. **Store the kits at 2 to 8°C before use, throw away the unspent kits.**
4. **Apart from the standard of kits, other components should not be refrigerated.**
5. **Please perform simple centrifugation to collect the liquid before use.**
6. **Apart from Stop Buffer and Concentrated Wash Buffer can be commonly used, the other components in the kits are specified. Do not mix or substitute reagents with those from other lots or other sources.**
7. **Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.**
8. **Mix the sample and all components in the kits adequately, and use clean plastainer to prepare wash buffer.**
9. **Both the sample and standard should be assayed in duplicate, and the sequence of the regents should be added consistently.**
10. **The kit should not be used beyond the expiration date.**
11. **The kit should be away from light when it is stored or incubated.**

12. **To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.**
13. **To avoid cross contamination, please use disposable pipette tips.**
14. **Please prepare all the kit components according to the requirement. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.**

Experiment Materials

1. **ELIASA (measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm)**
2. **Pipettes and pipette tips：0.5-10, 2-20, 20-200, 200-1000 μL**
3. **Microplate washer, Squirt bottle**
4. **Micro-oscillator**
5. **Deionized or double distilled water, graduated cylinder**
6. **Polypropylene Test tubes for dilution**

Reagent Preparation

1. **Bring all reagents to room temperature before use. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.**
2. **Wash buffer: 1:20 diluted with double distilled or deionized water before use.**

3. **Biotin-Conjugate antibody: 1:100 diluted with the Biotin-Conjugate antibody Dilutent (R2) before use, and the diluted solution should be used up within 30 min.**

Dilution Method

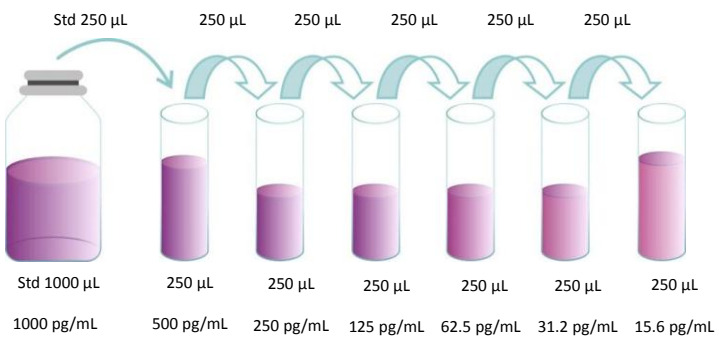
Strip	Concentrated Biotin-Conjugate antibody (1:100)	Testing dilution buffer (R2)
2	20	1980
4	40	3960
6	60	5940
8	80	7920
10	100	9900
12	120	11880

4. **Streptavidin-HRP: 1:100 diluted with the Streptavidin-HRP Diluent (R3) before use, and the diluted solution should be used up within 30 min.**

Dilution Method

Strip	Concentrated Streptavidin-HRP (1:100)	Testing dilution buffer (R3)
2	20	1980
4	40	3960
6	60	5940
8	80	7920
10	100	9900
12	120	11880

5. **Standard: Add standard/sample dilution (R1) 1mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (1000 pg/mL), then dilute according to the requirement (recommended concentration for standard curve: 1000, 500, 250, 125, 62.5, 31.25, 15.625, 0 pg/mL). Redissolved standard solution (1000 pg/mL), aliquot and store at -20°C— -70°C.**



Wash Method

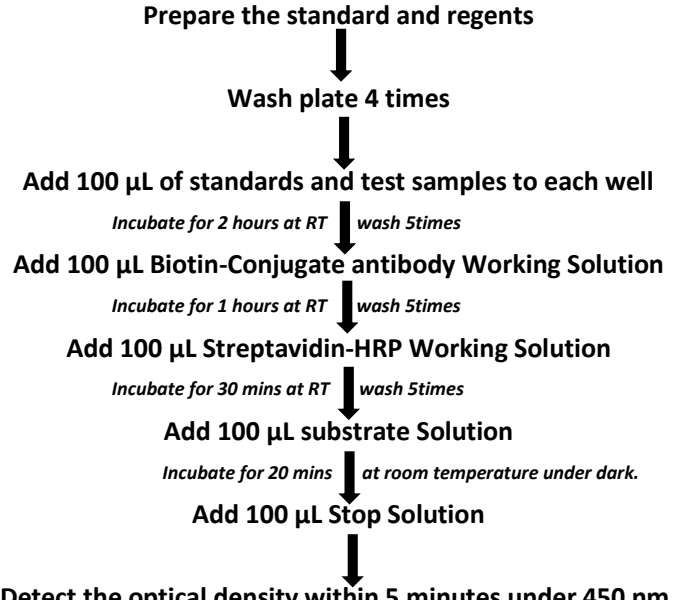
Automatic washer: Add wash buffer 300 µl/well, soak for about 10-20 seconds, and wash 5 times.
Washer: Throw all the solutions in the plate well, clean with absorbent paper, and then dispense wash buffer 300 µL/well, throw all the solutions in the plate well after holding 30 seconds, repeat 4 times.

Assay Procedure

1. **Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.**
2. **Add wash buffer 300 µL/well, aspirate each well after holding 30 seconds, repeating the process three times for a total of four washes. Then use enzyme-marked plate in a short time, do not let it dry.**
3. **Add 100µL Standard /Sample Diluent (R1) in blank well.**
4. **Apart from blank well, add 100 µl different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at room temperature (20 to 25°C)**
5. **Wash the plate 5 times as in step 2.**
6. **Prepare the Biotin-Conjugate antibody Working Solution 20 minutes early.**
7. **Add Biotin-Conjugate antibody diluent (R2) in blank well and Biotin-Conjugate antibody Working Solution in other wells (100 µL/well), cover with new adhesive strip provided, shake with Micro-oscillator (100 r/min). Incubate for 1 hours at room temperature (20 to 25°C)**
8. **Prepare the Streptavidin-HRP Working Solution 20 minutes early, place away from light at room temperature.**

9. **Wash the plate 5 times as in step 2.**
10. **Aspirate Streptavidin-HRP diluent (R3) in blank well and aspirate Streptavidin-HRP Working Solution in other wells (100 µL/well), cover with new adhesive strip provided, shake with Micro-oscillator (100 r/min). Incubate for 30 minutes at room temperature (20 to 25°C)**
11. **Warm-up the ELIASA.**
12. **Wash the plate 5 times.**
13. **Aspirate substrate Solution (100 µL/well). Incubate for 20 minutes at room temperature under dark.**
14. **Aspirate Stop Solution (100 µL/well), mix, determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.**

Assay Procedure Summary

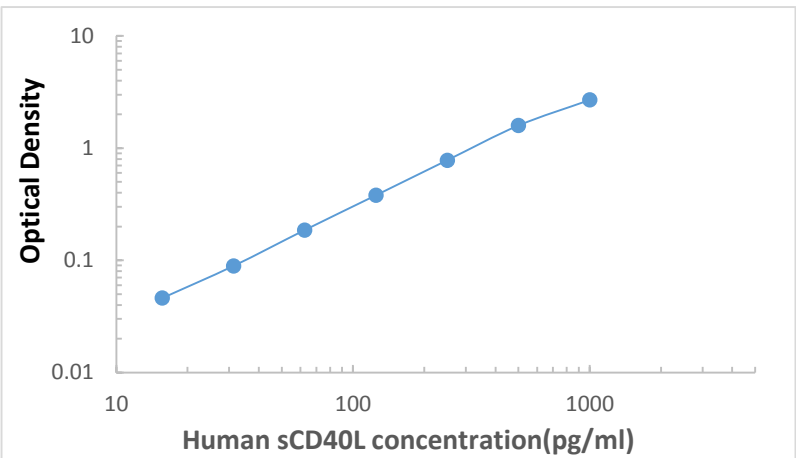


Calculation Of Results

1. **Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).**
2. **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 a log/log graph. The data may be linearized by plotting the log of the sCD40L concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.**
3. **If the detect result is higher than the standard curve's upper limit, then dilute samples, and the concentration read from the standard curve must be multiplied.**

Typical Data

Standard (pg/mL)	OD value		Average value	Correct value
0	0.06	0.062	0.061	---
15.62	0.106	0.109	0.107	0.046
31.25	0.146	0.154	0.150	0.089
62.5	0.258	0.236	0.247	0.186
125	0.425	0.457	0.441	0.380
250	0.864	0.826	0.845	0.780
500	1.634	1.668	1.651	1.590
1000	2.731	2.761	2.746	2.685



The standard curves are provided for demonstration only. A standard curve should be generated for each set of sCD40L assayed.

Sensitivity

The minimum detectable dose (MDD) of sCD40L ranged from 7 pg/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes both recombinant and natural human sCD40L. Use 50 ng/mL to do specificity assay. No significant cross-reactivity was observed with the following:

Recombinant human:

ANG

AG

BDNF

CD4

CD40

CNTF

CT-1

CTLA-4

Epo

Fas

CDNF

GITR

OPG

OSM

Precision

Intra-plate Precision

Repeat 20 times detection of 3 known concentration sample enzyme plate to evaluate the Intra-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	120	650	1300
Standard Deviation (SD)	5.1	22.7	53.3
Variable Coefficient CV (%)	4.3	3.5	4.1

Inter-plate Precision

Repeat 20 times detection of 3 known concentration sample enzyme plate to evaluate the Inter-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	125	750	1500
Standard Deviation (SD)	8.8	54.7	102
Variable Coefficient CV (%)	7.1	7.3	6.8

Recovery

Aspirate 3 different concentration of human sCD40L into healthy human serum and plasma, calculate the recovery.

Sample Form	Average Recover (%)	Range (%)
Serum	96	83-116
Plasma	99	86-108

Linearity Dilute

Aspirate high concentration of human sCD40L into 4 healthy human serum, dilute in the range of standard curve kinetics and evaluate the linearity.

Dilution	Average Value (%)	Range (%)
1:2	97	84-115
1:4	96	85-113
1:8	97	90-120
1:16	97	91-116

References

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