Datasheet for ABIN2472082

CD40 Ligand ELISA Kit

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Overview

Quantity:	96 tests
Target:	CD40 Ligand (CD40LG)
Binding Specificity:	Soluble
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	156.25-10000 pg/mL
Minimum Detection Limit:	156.25 pg/mL
Application:	ELISA

Product Details

Purpose:	This assay employs the quantitative sandwich enzyme immunoassay technique for quantitative detection.
Sample Type:	Serum, Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	SCD40L, Human
Sensitivity:	11.3 pg/mL
Components:	Plate, Standard, Diluent
Material not included:	Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.

Product Details

	Pipettes and pipette tips. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips. Multichannel micropipette reservoir. Beakers, flasks, cylinders necessary for preparation of reagents. Deionized or distilled water. Polypropylene test tubes for dilution.

Target Details

Target:	CD40 Ligand (CD40LG)
Alternative Name:	CD40L (CD40LG Products)
Background:	CD40 ligand (CD40L, CD154) is a member of the TNF superfamily of molecules and is a CD40L is a 39 kDa glycoprotein that is primarily expressed on activated T cells. 15-18 kDa soluble forms of CD40L (sCD40L) is produced by proteolytic cleavage. CD40L binds to CD40 on antigen-presenting cells (APC), which leads to many effects depending on the target cell type. CD40L acts as a costimulatory molecule and is particularly important on a subset of T cells called T follicular helper cells (TFH cells).
Gene ID:	959
NCBI Accession:	NM_000074
UniProt:	P29965
Pathways:	NF-kappaB Signaling, Production of Molecular Mediator of Immune Response, Cancer Immune Checkpoints

Application Details

Sample Volume:	100 µL
Assay Time:	3 - 4 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents and standards as directed. 2. Add 50 µL Assay Buffer to each well. 3. Add 50 µL Standard or sample per well within 15 minutes. 4. Add 50 µL Detect Antibody to each well. 5. Incubate for 2 hours at RT. 6. Aspirate and wash 6 times.

	7. Add 100 µL Substrate Solution to each well. Incubate for 10 - 30 minutes at RT. Protect from light. 8. Add 100 µL Stop Solution to each well. 9. Read at 450 nm within 30 minutes. Correction 570 or 630 nm
Reagent Preparation:	If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved. Washing Buffer (1x) Pour entire contents (50 mL) of the Washing Buffer (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with pure or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2 to 25 °C. Washing Buffer (1x) is stable for 30 days. Assay Buffer (1x) Pour the entire contents (5 mL) of the Assay Buffer (10x) into a clean 100 mL graduated cylinder. Bring to final volume of 50 mL with distilled water. Mix gently to avoid foaming. Store at 2 to 8 °C. Assay Buffer (1x) is stable for 30 days. Detect Antibody Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Detect Antibody solution with Assay Buffer (1x) in a clean plastic tube as needed. The diluted Detect Antibody should be used within 30 minutes after dilution. Sample Dilution If your samples have high sCD40L content, dilute serum/plasma samples with Assay Buffer (1x). For cell culture supernates, dilute with cell culture medium. Human sCD40L Standard Reconstitute Human sCD40L Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 40000 pg/ mL). Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions. Use polypropylene tubes. Human sCD40L ELISA Kit 7 For serum/plasma samples, mixing concentrated human sCD40L standard (150 µL) with 150 µL of Assay Buffer (1x) creates the high standard (20000 pg/ mL). Pipette 150 µL of Assay Buffer (1x) into each tube. Use the high standard to produce a 1:1 dilution series . Mix each tube thoroughly before the next transfer. Assay Buffer (1x) serves as the zero standard (0 pg/ mL). For cell culture supernates, mixing concentrated human sCD40L standard (150 µL) with 150 µL of cell culture medium creates the high standard (20000 pg/ mL). Pipette 150 µL of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ mL).
Sample Collection:	Cell Culture Supernates: Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ 20 °C for later use. Avoid repeated freeze thaw cycles. Serum:

	Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000 g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ 20 °C for later use. Avoid repeated freeze thaw cycles. Plasma: Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1000 g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at ≤ 20 °C for later use. Avoid repeated freeze thaw cycles. Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay. Dilution with Assay Buffer may be needed. Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. If samples are to be run within 24 hours, they may be stored at 2 to 8 °C. For longer storage, aliquot samples and store frozen at 20 °C to avoid loss of bioactive human sCD40L. Avoid repeated freeze thaw cycles
Assay Procedure:	Bring all reagents and samples to room temperature before use. 1. Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections. 2. Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use. 3. Add 300 µL Washing Buffer (1x) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1x). Use the microwell strips immediately after washing. Do not allow wells to dry. 4. Add 50 µL of Assay Buffer (1x) to each well. 5. Add 50 µL of Standard or sample to each well. Ensure reagent addition is uninterrupted and completed within 15 minutes. 6. Add 50 µL of Detect Antibody to each well. 7. Seal the plate with an adhesive film. Incubate at room temperature (18 to 25 °C) for 2 hours on a microplate shaker set at 100 rpm. 8. Aspirate each well and wash by filling each well with 300 µL Washing Buffer (1x), repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining Washing Buffer (1x) by aspirating or decanting. Invert the plate and tap it against clean paper towels. 9. Add 100 µL of Substrate Solution to each well. Incubate for 10 - 30 minutes at room temperature. Protect from light. 10. Add 100 µL of Stop Solution to each well. The color will turn

Application Details

	yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing. 11. Measure the optical density value within 30 minutes by 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. Reading directly at 450 nm without correction may generate higher concentration than true value.
Calculation of Results:	Average the duplicate optical density readings for each standards and sample, then subtract the average optical density value of the zero standard. Standard Concentration as horizontal axis, optical density (OD) Value as the vertical axis, regressing the data and create a standard curve using computer software. The data may be linearized by plotting the log of the sCD40L concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. Note: The finally concentration of top standard is 10000 pg/mL. If instruction in this protocol have been followed samples have been diluted by 1:1 ratio (50 µL sample + 50 µL Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (x2). If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Assay Precision:	Intra-assay precision (precision within an assay): Three serum-based and buffer-based samples of known concentration were tested twenty times on one plate to assess intra-assay precision. Inter-assay precision (precision between assays): Three serum-based and buffer-based samples of known concentration were tested in six separate assays to assess inter-assay precision. Spike recovery: The spike recovery was evaluated by spiking 3 levels of human sCD40L into five health human serum samples. The un-spiked serum was used as blank in these experiments. The recovery ranged from 88 % to 110 % with an overall mean recovery of 101 %.
Restrictions:	For Research Use only

Handling

Buffer:	Buffer contains: 0.02 % sodium azide
Preservative:	Sodium azide

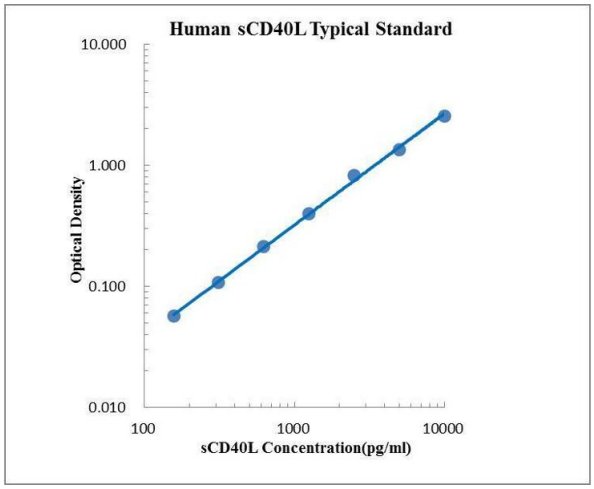
Handling

Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Intended for research use only and are not for use in diagnostic or therapeutic procedures. Treat all chemicals with caution because they can be potentially hazardous. It is recommended that this product is handled only by persons who have been trained in laboratory techniques and in accordance with the principles of good laboratory practice. Wear personal protection equipment such as laboratory coat, safety glasses and gloves. Avoid direct contact with skin or eyes. Wash immediately with water in the case of contact with skin or eyes. Avoid contact of skin or mucous membranes with kit reagents or specimens. See material safety data sheet(s) for specific advice. Pure water or deionized water must be used for reagent preparation. The Stop Solution provided with this kit is an acid solution. Wear personal protection equipment with caution. Do not expose kit reagents to strong light during storage and incubation. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens. Avoid contact of substrate solution with oxidizing agents and metal. Avoid splashing or generation of aerosols. Use disposable pipette tips and/or pipettes to avoid microbial or cross-contamination of reagents or specimens which may invalidate the test. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent. Exposure to acid inactivates the HRP and antibody conjugate. Substrate solution must be warmed to room temperature prior to use. Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5 °C. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite. All reagents including microplate, samples, standards and working solution should be warmed to room temperature before use. To obtain accurate results, using adhesive film to seal the plate during incubation is suggested. It is recommended that all samples and standards be assayed in duplicate. Avoid foaming when mixing or reconstituting solutions containing protein. To avoid cross-contamination, use separate reservoirs for each reagent and change pipette tips

Handling

	between each standard, sample and reagent. When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision. When pipetting reagents, maintain a consistent order of addition from well-to-well. Keep Substrate solution protected from direct strong light. Substrate Solution should turn to gradations of blue after a proper color development. Read absorbance within 30 minutes after adding stop solution. Take care not to scratch the inner surface of the microwells.
Storage:	4 °C
Storage Comment:	Store kit reagents between 2 and 8 °C. Immediately after use remaining reagents should be returned to cold storage (2 to 8 °C). Expiry of the kit and reagents is stated on labels. Expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling. Unopened Kit: Store at 2 - 8 °C (See expiration date on the label). Opened/Reconstituted Reagents: Up to 1 month at 2 - 8 °C. Reconstituted Standard: Up to 1 month at ≤ -20 °C in a manual defrost freezer. Avoid repeated freeze-thaw cycles. Microplate Wells: Up to 1 month at 2 - 8 °C. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity. Provided this is within the expiration date of the kit

Images



ELISA

Image 1.