Datasheet for ABIN624956

CD40 ELISA Kit

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Overview

Quantity:	96 tests
Target:	CD40
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	50-40000 pg/mL
Minimum Detection Limit:	50 pg/mL
Application:	ELISA

Product Details

Purpose:	Human CD40 (TNFRSF5) ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin, BDNF, BLC, ENA-78, FGF- 4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin (OB), MCP-1, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, MMP-1, -2, -3, -10, PARC, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.
Sensitivity:	< 50 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments

Product Details

	• Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	CD40
Alternative Name:	CD40 (CD40 Products)
Background:	The Human CD40 (TNFRSF5) ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human CD40 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human CD40 coated on a 96-well plate. Standards and samples are pipetted into the wells and CD40 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human CD40 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CD40 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	958

Target Details

UniProt:	P25942
Pathways:	NF-kappaB Signaling, Cellular Response to Molecule of Bacterial Origin, M Phase, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Cancer Immune Checkpoints

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, 1x Assay Diluent D (Item K) should be used for dilution of serum/plasma/ culture supernatants/urine. Suggested dilution for normal serum/plasma: 2 fold*. *Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent D (Item K) and Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µL 1x Assay Diluent D (Item K) into Item C vial to prepare a 100 ng/mL standard solution. Dissolve the powder thoroughly by a gentle mix. Add 180 µL CD40 standard from the vial of Item C, into a tube with 270 µL 1x Assay Diluent D to prepare a 40,000 pg/mL standard solution. Pipette 400myl 1x Assay Diluent D into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. 1x Assay Diluent D serves as the zero standard (0 pg/mL). 200 µL 200 µL 200 µL 200 µL 200 µL 180 µL standard + 270 µL 200myl 40,000 13,333 4,444 1,481 493.8 164.6 54.87 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL

	5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent Band used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 500-fold with 1x Assay Diluent B (Item E). For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 20 µL of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a final 500 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette 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. 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Application Details

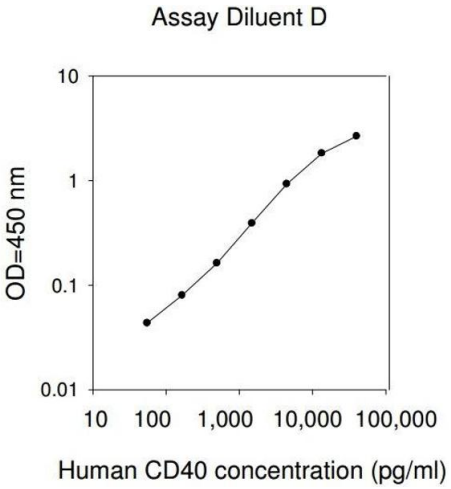
	Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent D Human CD40 concentration (pg/mL) O D =4 50 n m 0.01 0.1 1 10 10 100 1,000 10,000 100,000 Sensitivity: The minimum detectable dose of CD40 is typically less than 50 pg/mL. Recovery: Recovery was determined by spiking various levels of CD40 into normal human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 114.7 104-124 Plasma 90.19 78-100 Cell culture media 92.27 80-100 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 116.0 124.2 110.0 Range (%) 105-126 113-134 100-128 1:4 Average % of Expected 121.5 132.2 119.4 Range (%) 110-132 122-143 108-131 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Idriss, Blann, Sayed, Gaber, Hassen, Kishk: "Circulating Endothelial Cells and Platelet Microparticles in Mitral Valve Disease With and Without Atrial Fibrillation." in: **Angiology**, Vol. 66, Issue 7, pp. 631-7, (2015) (PubMed).



ELISA

Image 1.