Datasheet for ABIN625116

CD40 ELISA Kit

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Overview

Quantity:	96 tests
Target:	CD40
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	3-1500 pg/mL
Minimum Detection Limit:	3 pg/mL
Application:	ELISA

Product Details

Purpose:	Mouse CD40 (TNFRSF5) ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Serum, Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fractalkine, GCSF, GM-CFS, IFN-gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, MIG, MIP- 1alpha, MIP-1 gamma, MIP-2, MIP-3 beta, MIP-3 alpha, PF-4, P-Selectin, RANTES, SCF, SDF-1 alpha, TARC, TCA-3, TECK, TIMP-1, TNF-alpha, TNF RI, TNF RII, TPO, VCAM-1, VEGF.
Sensitivity:	< 3 pg/mL

Product Details

Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • 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 Mouse CD40 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse CD40/TNFRSF5 in serum, plasma and cell culture supernatants. This assay employs an antibody specific for mouse 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-mouse 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:	21939

Target Details

UniProt:	P27512
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 samples3 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: Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of cell culture supernatants. Suggested dilution for normal serum/plasma: 3 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 B should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 15 µL CD40 standard (50 ng/mL) from the vial of Item C, into a tube with 485 µL Assay Diluent A or 1x Assay Diluent B to prepare a 1,500 pg/mL standard solution. Pipette 400 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the 1,500 pg/mL standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL). 15 µL standard + 485 µL 200 µL 200 µL 200 µL 200 µL

	200 µL 200myl 1,500 500 166.7 55.56 18.52 6.17 2.06 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 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 B and 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 200 fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 50 µL of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a 200-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

Application Details

	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. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Mouse CD40 concentration (pg/mL) 1 10 100 1000 10000 O D =4 50 n m 0.01 0.1 1 10 Assay Diluent B Mouse CD40 concentration (pg/mL) 1 10 100 1000 10000 O D =4 50 n m 0.01 0.1 1 10 Sensitivity: The minimum detectable dose of CD40 is typically less than 3 pg/mL. Recovery: Recovery was determined by spiking mouse CD40 into mouse serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 77.26 71-87 Plasma 76.68 69-87 Cell culture media 84.23 74-93 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 100.9 102.5 108.2 Range (%) 91-110 93-110 97-117 1:4 Average % of Expected 104.9 102.0 108.0 Range (%) 92-112 90-109 96-17 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:	Mason, Jacob, Kubant, Walter, Bellamine, Jacoby, Mizuno, Malinski: "Effect of enhanced glycemic control with saxagliptin on endothelial nitric oxide release and CD40 levels in obese rats." in: **Journal of atherosclerosis and thrombosis**, Vol. 18, Issue 9, pp. 774-83, (2012) (PubMed).

ELISA

Image 1.