Version 28-04/24

User's Manual

Mouse CD48 ELISA Kit

REF **DEIASL191**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit is intended for the quantitation of Mouse Cd48 concentrations in cell culture supernates, cell lysates, serum and plasma (heparin, EDTA).

General Description

CD48 antigen (Cluster of Differentiation 48), also known as BLAST-1 or SLAMF2, is a protein that in humans is encoded by the CD48 gene. CD48 is a member of the CD2 subfamily of the immunoglobulin superfamily (IgSF) which includes SLAM (signaling lymphocyte activation molecules) proteins, such as CD84, CD150, CD229 and CD244. It is mapped to 1q23.3. CD48 is found on the surface of lymphocytes and other immune cells, dendritic cells and endothelial cells, and participates in activation and differentiation pathways in these cells. The encoded protein does not have a transmembrane domain, however, but is held at the cell surface by a GPI anchor via a C-terminal domain which maybe cleaved to yield a soluble form of the receptor.

Principles of Testing

The Mouse Cd48 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Cd48 with a 96-well strip plate that is pre-coated with antibody specific for Cd48. The detection antibody is a biotinylated antibody specific for Cd48. The capture antibody is monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Mouse Cd48 with immunogen: Expression system for standard: NSO; Immunogen sequence: F23-S217. The kit is analytically validated with ready to use reagents.

To measure Mouse Cd48, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Mouse Cd48 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Mouse Cd48 in the sample.

Reagents And Materials Provided

1. Anti-Mouse CD48 Pre-coated 96-well strip microplate: 12 strips of 8 wells
2. Mouse CD48 Standard: 2vial, 10ng/tube
3. Mouse CD48 Biotinylated antibody (100x): 130 µl
4. Avidin-Biotin-Peroxidase Complex (100x): 130 µl
5. Sample Diluent: 30ml
6. Antibody Diluent: 12ml
7. Avidin-Biotin-Peroxidase Diluent: 12ml
8. Color Developing Reagent (TMB): 10ml

9. Stop Solution: 10ml
10. Plate Sealers: 4
11. Wash Buffer: 1vial, powder pack for 1000ml

Materials Required But Not Supplied

1. Microplate Reader capable of reading absorbance at 450nm.
2. Automated plate washer (optional)
3. Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
4. Multichannel pipettes are recommended for large amount of samples.
5. Deionized or distilled water.
6. 500ml graduated cylinders.
7. Test tubes for dilution.
8. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H_2O

Preparation of 0.01 M PBS: Add 8.5g Nacl, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6.

Storage

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)

Specimen Collection And Preparation

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

1. Cell culture supernatants: Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
2. Serum: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
3. Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.
4. Cell lysates: Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10000 X g for 5 min. Collect the supernatant.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay. It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Reagent Preparation

Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C.

1. Wash buffer: Prepare standard 1X PBS as wash buffer. Wash buffer can be prepared in-house or purchased from Creative Diagnostics. Preparation of wash buffer: Add 8.5g NaCl, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6.
2. Biotinylated Anti-Mouse CD48 antibody: It is recommended to prepare this reagent immediately prior to use by diluting the Mouse CD48 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
3. Avidin-Biotin-Peroxidase Complex: It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-BiotinPeroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
4. Human CD48 Standard: It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Mouse CD48 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

(1) Number tubes 1-8. Final Concentrations to be Tube # 1 –4000pg/ml, #2 –2000pg/ml, #3 – 1000pg/ml, #4 – 500pg/ml, #5 – 250pg/ml, #6 – 125pg/ml, #7 – 62.5pg/ml, #8 – Sample Diluent serves as the zero standard (0pg/ml).

(2) To generate standard #1, add 400µl of the reconstituted standard stock solution of 10ng/ml and 600µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.

(3) Add 300 µl of sample diluent to tubes # 2-7.

(4) To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.

(5) To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.

(6) Continue the serial dilution for tube #4-7.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).

5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Mouse CD48 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.

a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 2 additional times.

9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.

a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 2 additional times.

11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Calculation

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logisticcurve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

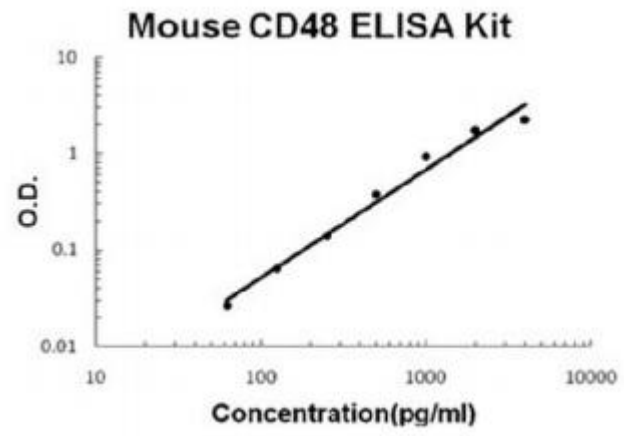
For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution

factor.

Typical Standard Curve

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	62.5	125	250	500	1000	2000	4000
O.D.	0.000	0.026	0.063	0.140	0.379	0.917	1.707	2.273



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Precision

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess interassay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/ml)	91	816	1332	95	736	1273
Standard deviation	4	34.27	87.91	5.03	41.21	98.02
CV(%)	4.4%	4.2%	6.6%	5.3%	5.6%	7.7%

Detection Range

62.5pg/ml-4000pg/ml

Sensitivity

The sensitivity of this kit < 10pg/ml.

*The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20)

blank wells and calculating the corresponding concentration.

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	91	79	95	86	87	5.97	6.8%
Sample 2	816	795	773	755	784	22.93	2.9%
Sample 3	1332	1482	1540	1536	1472	84.28	5.7%

Precautions

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.