



**EAGLE**  
BIOSCIENCES

# **Human MCAM/CD146 ELISA Kit**

Catalog Number:

**MCM31-K01 (1 x 96 wells)**

*For Research Use Only. Not for use in diagnostic procedures.*

*v. 1.0*

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## INTENDED USE

The Eagle Biosciences Human MCAM/CD146 ELISA Kit is intended for the quantitation of Human MCAM concentrations in cell culture supernates and serum. The Eagle Biosciences Human MCAM/CD146 ELISA Kit is for research use only and not for diagnostic or therapeutic procedures.

## INTRODUCTION

CD146 (cluster of differentiation 146) also known as the melanoma cell adhesion molecule (MCAM) or cell surface glycoprotein MUC18, is a 113kDa cell adhesion molecule currently used as a marker for endothelial cell lineage. In humans, the CD146 protein is encoded by the MCAM gene. By radiation hybrid analysis, it mapped the CD146 gene to chromosome 11q23.3. It is a member of the immunoglobulin superfamily, is homologous to several cell adhesion molecules and is associated with tumor progression and the development of metastasis in human malignant melanoma. CD146 has been demonstrated to appear on a small subset of T and B lymphocytes in the peripheral blood of healthy individuals. The CD146+ T cells display an immunophenotype consistent with effector memory cells and have a distinct gene profile from the CD146- T cells. CD146 has been seen as a marker for mesenchymal stem cells isolated from multiple adult and fetal organs, and its expression may be linked to multipotency; mesenchymal stem cells with greater differentiation potential express higher levels of CD146 on the cell surface.

## PRINCIPLE OF THE ASSAY

The Human MCAM Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human MCAM with a 96-well strip plate that is pre-coated with antibody specific for MCAM. The detection antibody is a biotinylated antibody specific for MCAM. The capture antibody is a monoclonal antibody from mouse, the detection antibody is a biotinylated polyclonal antibody from goat. The kit contains recombinant Human MCAM with immunogen: Expression system for standard: NSO, Immunogen sequence: V24-G559. The kit is analytically validated with ready to use reagents. To measure Human MCAM, 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 unbound 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 Human MCAM 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 Human MCAM in the sample.

<b>Reactive Species</b>	Human
<b>Size</b>	96 wells/kit, with removable strips
<b>Description</b>	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human MCAM. 96wells/kit, with removable strips.
<b>Sensitivity</b>	<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.
<b>Detection Range</b>	62.5pg/ml-4000pg/ml
<b>Storage Instructions</b>	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)



## TECHNICAL DETAILS

### Capture/Detection Antibodies

The capture antibody is monoclonal antibody from mouse, the detection antibody is a biotinylated polyclonal antibody from goat.

### Specificity

Natural and recombinant Human MCAM

### Immunogen

Expression system for standard: NSO, Immunogen sequence: V24-G559

### Cross Reactivity

There is no detectable cross-reactivity with other relevant proteins.

## NOTICE BEFORE APPLICATION

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.

## KIT COMPONENTS/MATERIALS PROVIDED

Description	Quantity	Volume
Anti-Human MCAM Precoated 96-well strip microplate	1	12 strips of 8 wells
Human MCAM Standard	2	4ng/tube
Human MCAM Biotinylated antibody (100x)	1	130 $\mu$ l
Avidin-Biotin-Peroxidase Complex (100x)	1	130 $\mu$ l
Sample Diluent	1	30ml
Antibody Diluent	1	12ml
Avidin-Biotin-Peroxidase Diluent	1	12ml
Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Wash Buffer Powder	1	Pack
Plate Sealers	4	Piece

## REQUIRED MATERIALS THAT ARE NOT SUPPLIED

Microplate Reader capable of reading absorbance at 450nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5  $\mu$ l through 1 ml volumes of aqueous solutions.

Multichannel pipettes are recommended for large amount of samples.

Deionized or distilled water.

500ml graduated cylinders.

Test tubes for dilution.

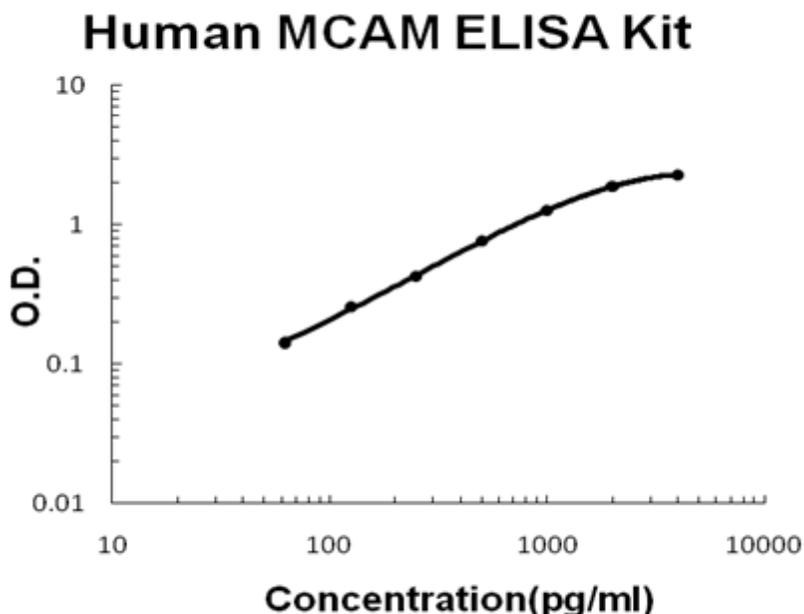


**STANDARD CURVE EXAMPLE**

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.	0.004	0.142	0.258	0.426	0.761	1.266	1.869	2.264

**Human MCAM/CD146 ELISA Kit Standard Curve**



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

**INTRA/INTER ASSAY VARIABILITY**

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 inter-assay precision.

**Intra-Assay Precision**

Sample	1	2	3
N	16	16	16
Mean(pg/ml)	143	548	1021
Standard Deviation	6	23.56	62.28
CV (%)	4.2%	4.3%	6.1%



### Inter-Assay Precision

Sample	1	2	3
N	24	24	24
Mean(pg/ml)	131	561	1060
Standard Deviation	7.07	31.97	80.56
CV (%)	5.4%	5.7%	7.6%

### 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	143	158	162	156	154	7.11	4.6%
Sample 2	548	529	527	526	532	9.01	1.6%
Sample 3	1021	1095	1036	1044	1049	27.81	2.6%

\*number of samples for each test n=16.

### PREPARATION BEFORE THE EXPIREMENT

Item	Preparation
All reagents	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.
Wash Buffer	Dissolve the wash buffer powder in 1000ml of water to make 1X PBS wash buffer.
Biotinylated Anti-Human MCAM antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human MCAM 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.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase 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.
Human MCAM Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 4ng of lyophilized Human MCAM standard for each experiment. Gently spin the vial prior to use.



	Reconstitute the standard to a stock concentration of 4ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

### DILUTION OF HUMAN MCAM STANDARD

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 – 0.0 (Blank).
2. For standard #1, add 1000µl of undiluted standard stock solution to tube #1.
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.
7. Tube #8 is a blank standard to be used with every experiment.

### SAMPLE PREPARATION AND STORAGE

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

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
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.

### 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.

### ASSAY PROTOCOL

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  $\mu$ l of the standard, samples, or control per well. Add 100  $\mu$ l of the sample diluent buffer into the control well (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  $^{\circ}$ 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  $\mu$ l of the prepared 1x Biotinylated Anti-Human MCAM antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37 $^{\circ}$ 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  $\mu$ l 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  $\mu$ l 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 $^{\circ}$ 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  $\mu$ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
  - c. Repeat steps a-b 4 additional times.
11. Add 90  $\mu$ l 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 $^{\circ}$ 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  $\mu$ l 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.

## DATA ANALYSIS

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](http://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.

### **Warranty Information**

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.