MOUSE ENDOTHELIAL-CELL SPECIFIC MOLECULE-1 (ESM-1)/ENDOCAN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE ESM-1 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND URINE

PURCHASE INFORMATION:

ELISA NAME	MOUSE ESM-1 /ENDOCAN ELISA
Catalog No.	SK00318-04
Lot No.	
Formulation	96 T
Standard range	78-5000 pg/mL
Sensitivity	30 pg/mL
Sample Volume	50 µL
Sample Type	Cell Culture Supernates, Serum, Urine
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Mouse ESM-1 (50KD) HEK293 derived at 100%.
Calibration	Mouse ESM-1 (50KD) HEK293 derived recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
Storage	2 - 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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DESCRIPTION

This Mouse ESM-1/Endocan ELISA Kit contains the necessary components required for the quantitative measurement of recombinant mouse ESM-1 (50KD) from transfected HEK293 culture supernates, mouse serum and tissue homogenates in a sandwich ELISA format. This ELISA Kit may not detect the mouse ESM1 derived from E. Coli. There were no detectable mouse ESM1 levels found in BALB/C mouse serum samples.

This immunoassay contains recombinant mouse ESM-1 (50 KD) HEK293 derived and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural ESM-1 in samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for mouse ESM-1. The capture antibody can bind to the mouse ESM-1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against mouse ESM-1 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of mouse ESM-1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
ESM-1 Microplate - 96 well microplate coated with an antibody specific for mouse ESM-1.	**318-04-01**	**1 plate**
ESM-1 Standard – refer to package label of lyophilized recombinant mouse ESM-1.	**318-04-02**	**1 vial**
Detection Antibody Concentrate – refer to package label, 10-fold concentrate of lyophilized biotinylated antibody against mouse ESM-1.	**318-04-03**	**1 vial**
Positive Control - one vial of lyophilized recombinant mouse ESM-1.	**318-04-04**	**1 vial**
Streptavidin-HRP Conjugate - 80 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered solution with preservative.	**DB101**	**1 bottle**
Antibody Diluent Solution – 8 mL of buffered solution with preservative.	**DB102**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered solution with preservative.	**DB01**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution -11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8° C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8° C for up to 8 months.
Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Centrifuge and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION

Cell culture samples may require dilution. Mouse serum samples may require dilution .A pretest will help determine the optimal dilution factor for the samples if needed. **Optimal dilutions should be determined by each laboratory for each application.**
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

ESM-1 Standard - Reconstitute the ESM-1 standard with refer to package label of Dilution Buffer. This reconstitution produces a stock solution of refer to package label. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 150 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **5000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	Refer to label	20000 pg/ml
# 1	80 µl of stock	240 µl	5000 pg/ml
# 2	150 µl of 1	150 µl	2500 pg/ml
# 3	150 µl of 2	150 µl	1250 pg/ml
# 4	150 µl of 3	150 µl	625 pg/ml
# 5	150 µl of 4	150 µl	312.5 pg/ml
# 6	150 µl of 5	150 µl	156 pg/ml
# 7	150 µl of 6	150 µl	78 pg/ml

Positive Control – Reconstitute the Positive Control with refer to package label Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C ~ -70° C.
Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to package label **Antibody Diluent Solution (DB102)** to produce a 10-fold concentrated stock solution. Pipette 5.4 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 0.6 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 5.94 mL of **HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 60 µl of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light). DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 50 µL per well of Dilution Buffer to Blank wells.
4. Add 50 µL of standard dilutions, samples, or positive control per well. Cover with plate sealer. For cell culture media samples, incubate for 2 hours on microplate shaker at room temperature. For mouse serum samples, may incubate for overnight at 4 °C.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, 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.
6. Add 50 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 50 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 10-15 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

Protein	Cross-reactivity
Mouse ESM-1 HEK293 (50KD) derived	100%
Mouse ESM-1 E. Coli derived	0

TYPICAL DATA

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

MOUSE ESM-1 (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.117)
78	0.020
156	0.042
312.5	0.092
625	0.189
1250	0.389
2500	0.812
5000	1.757

There were no detectable mouse ESM1 levels found in BALB/C mouse serum and EDTA plasma samples.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 50 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.

Aspirate and wash 4 times.

Add 50 µl Detection Antibody working solution to each well. For cell culture media samples, Incubate 2 hours on the plate shaker at RT. For mouse serum samples, may incubate for overnight at 4 °C.

Aspirate and wash 4 times.

Add 50 µl Streptavidin-HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light**.

Aspirate and wash 4 times.

Add 100 µl Substrate solution to each well. Incubate 12-15 min on plate shaker at RT. **Protect from light**.

Add 100 µl Stop Solution to each well. Read 450nm within 15 min.