

HUMAN ENDOTHELIAL-CELL SPECIFIC MOLECULE-1 (ESM-1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ESM-1 CONCENTRATIONS IN SERUM AND EDTA PLASMA



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

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

PRODUCT INFORMATION:

ELISA NAME	HUMAN ESM-1 ELISA
Catalog No.	SK00318-06
Lot No.	
Formulation	96 T
Standard range	3.125-200 ng/mL
Sensitivity	500 pg/mL
Sample Volume	100 µL
Sample Type	Serum and EDTA Plasma
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human ESM-1
Calibration	Human ESM-1 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.	

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DESCRIPTION

This Human ESM-1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human ESM-1 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human ESM-1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural ESM-1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human ESM-1. The capture antibody can bind to the human ESM-1 in the standard and samples. After washing the plate of any unbound substances, another biotinylated monoclonal antibody against human 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 human ESM-1 bound in the standard dilutions 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 polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against ESM-1.	318-06-01	1 plate
ESM-1 Standard – 200 ng/vial of recombinant human ESM-1 in a buffered protein base with preservative; lyophilized.	318-06-02	1 vial
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of the biotinylated monoclonal antibody against ESM-1 with preservative; lyophilized.	318-06-03	1 vial
Positive Control - one vial of recombinant human ESM-1; lyophilized.	318-06-04	1 vial
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin HRP conjugate with preservative.	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	DB06	1 bottle
Antibody & HRP Dilute Solution - 24 mL of buffered protein based solution with preservative.	DB08	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) and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. ARIG-HRP Conjugate 100-fold concentrated solution (**protect from light**) and other components may be stored at 2 – 8° C for up to 8 months.

Microplate Wells: Return unused wells 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

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. 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

Optimal dilutions should be determined by each laboratory for each application with a pretest.

Use polypropylene test tubes.

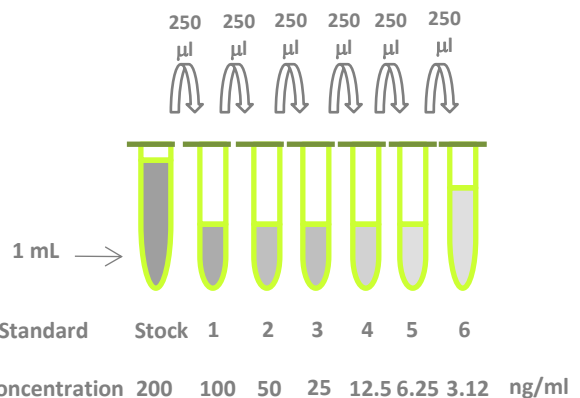
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

ESM-1 Standard - Reconstitute the ESM-1 Standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 200 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **200 ng/mL** standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 µl	200 ng/ml
# 1	250 µl of stock	250 µl	100 ng/ml
# 2	250 µl of 1	250 µl	50 ng/ml
# 3	250 µl of 2	250 µl	25 ng/ml
# 4	250 µl of 3	250 µl	12.5 ng/ml
# 5	250 µl of 4	250 µl	6.25 ng/ml
# 6	250 µl of 5	250 µl	3.125 ng/ml



Positive Control – Reconstitute the Positive Control with 1 mL 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 1.05 mL of Antibody & HRP Dilute Solution to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody & HRP Dilute Solution into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin -HRP Conjugate - Pipette 11.88 mL of Antibody & HRP Dilute Solution into a 15 mL centrifuge tube and transfer 120 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**).

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 and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 μL of Dilution Buffer to Blank wells.
4. Add 100 μL of Standard dilutions in reverse order of serial dilution, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 100 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 100 μL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 45 min 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 5-10 minutes on a 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.

TYPICAL DATA

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

STANDARD (ng/mL)	Average OD450 (Corrected)*
Blank	0 (0.124)
3.125	0.029
6.25	0.052
12.5	0.119
25	0.262
50	0.549
100	1.023
200	1.898

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human ESM-1	100
Human HGF	0
Human HGF-alpha	0
Human VEGF-R1	0
Human TNF-alpha	0
Human IL-6	0
Human CD36	0
Human VEGF	0
Human IL-1beta	0
Human IL-1RN	0
Mouse ESM-1	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 5-10 min on plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.