HUMAN SOLUBLE ENDOTHELIAL PROTEIN C RECEPTOR (sEPCR) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN sEPCR CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND EDTA PLASMA

PRODUCT INFORMATION:

ELISA NAME	HUMAN sEPCR ELISA
Catalog No.	SK00507-01
Lot No.	
Formulation	96 T
Standard range	0.312 - 20 ng/mL
Sensitivity	35 pg/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA Plasma and Cell Culture Supernates
Pretreatment	May be needed to release Protein C
Specificity	Human sEPCR
Calibration	Human sEPCR recombinant (HEK293 derived)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 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 Human sEPCR ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human sEPCR from cell culture supernates, serum and plasma in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human sEPCR. The capture antibody can bind to the human sEPCR in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human sEPCR 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 sEPCR 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
sEPCR Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sEPCR.	**507-01-01**	**1 plate**
sEPCR Standard – 40 ng/vial of recombinant human sEPCR in a buffered protein base with preservative; lyophilized.	**507-01-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated antibody against sEPCR with preservative; lyophilized.	**507-01-03**	**1 vial**
Positive Control - one vial of recombinant human sEPCR; lyophilized.	**507-01-04**	**1 vial**
Streptavidin HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 30 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody & HRP Diluent Solution – 30 mL of buffered protein based solution with preservative.	**DB08A**	**1 bottle**
Sample Pretreatment Solution A - 10 mL of activation solution.	**PTS06**	**1 bottle**
Sample Pretreatment Solution B – 10 mL of neutralization solution.	**PTS07**	**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**
Plastic Pouch	**P01**	**1**

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 **(protect from light)** and other components may be stored at 2 - 8° C for up to 8 months. Do not freeze TMB substrate solution.
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, sample pretreatment solution A because it contains diluted hydrochloric acid. Care should be taken while handling Sample Pretreatment Solution B in this kit to avoid contact with skin or eyes because it contains diluted sodium hydroxide. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
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.

ACTIVATION PROCEDURE

Samples may need to be pretreated to release Protein C. **DO NOT ACTIVATE THE STANDARD.**

1. To 100 µL sample add 50 µL **Sample Pretreatment Solution A**. Mix well.
2. Incubate 10 minutes at room temperature.
3. Add 50 µL of **Sample Pretreatment Solution B** (final pH should be 5.0 – 6.0, adjust accordingly). Mix well and assay immediately after neutralization.

Note: 1) Sample results must be multiplied by the dilution factor (2 if strictly follow activation procedure, if not, need to multiply by its dilution factor). If samples generate values higher than the highest standard, dilute the samples after activation with Dilution Buffer (DB01) and repeat the assay.
Use polypropylene tubes.

SAMPLE PREPARATION

Serum or EDTA plasma samples may require a 10-fold dilution after activation procedure (final dilution factor is 20). A suggested 10-fold dilution is 30 µL sample after activation procedure + 270 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**
Use polypropylene test tubes.

REAGENT PREPARATION

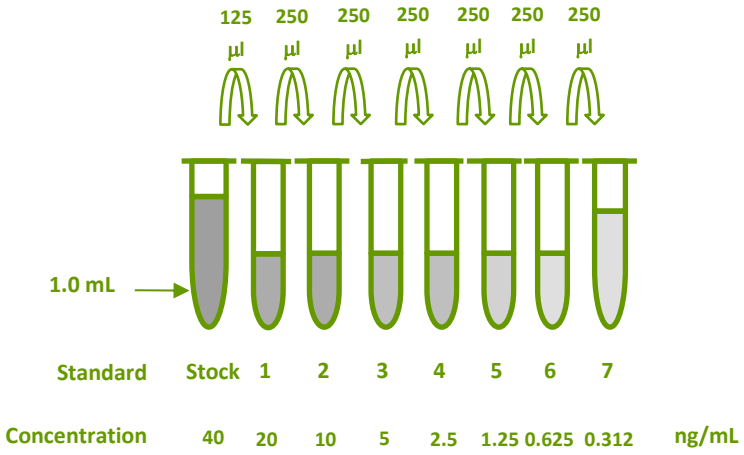
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm 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.

sEPCR Standard - Reconstitute the sEPCR standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 40 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 #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **20 ng/mL** standard serves as the high standard.

The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 µl	40 ng/ml
# 1	250 µl of stock	250 µl	20 ng/ml
# 2	250 µl of 1	250 µl	10 ng/ml
# 3	250 µl of 2	250 µl	5 ng/ml
# 4	250 µl of 3	250 µl	2.5 ng/ml
# 5	250 µl of 4	250 µl	1.25 ng/ml
# 6	250 µl of 5	250 µl	0.625 ng/ml
# 7	250 µl of 6	250 µl	0.313 ng/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C or -70° C.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody & HRP Diluent Solution (DB08A)** to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of **Antibody & HRP Diluent Solution (DB08A)** into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **Antibody & HRP Diluent Solution (DB08A)** 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, **samples**, 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 **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 12-17 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.

The concentration read from the standard curve need to be multiplied by its dilution factor (if samples were activated and/or diluted).

SPECIFICITY

No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY (%)
Human sEPCR (HEK293)	100
Mouse sEPCR	0
Human Coagulation Factor X	0
Human Thrombomodulin	0
Human Active Coagulation Factor XIV	0

TYPICAL DATA

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

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (0.069)
0.313	0.047
0.625	0.093
1.25	0.180
2.5	0.331
5	0.652
10	1.217
20	2.068

- Lot:
- Positive Control: 1.7-5 ng/mL

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or 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 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 12-17 min on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.