HUMAN SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE (SPARC)/ OSTEONECTIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF SPARC CONCENTRATIONS IN SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA Name	Human SPARC/OSTEONECTIN ELISA
Catalog No.	SK00766-01
Lot No.	
Formulation	96 T
Standard range	0.128-400 ng/mL
Dynamic range	0.128-400 ng/ml
Sensitivity	0.128 ng/mL
Sample Volume	50 µl
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma
Specificity	Human SPARC
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8°C

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

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INTRODUCTION

Human SPARC ELISA employs the quantitatively competitive enzyme immunoassay technique in which human SPARC present in samples compete with a fixed amount of biotinylated human SPARC for sites on an antibody specific against human SPARC. During the incubation, the standard and samples bound to the anti-human SPARC IgG pre-coated onto the microplates. The biotinylated SPARC competitively bound to antibody specific to SPARC. Following a wash to remove any unbound standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution is added. The intensity of the color measured is in inverse proportion to the amount of human SPARC bound in the initial step. The sample values are then read off the standard curve.

Human SPARC ELISA has been shown to accurately quantify the recombinant and natural human SPARC. Results obtained using natural human SPARC showed dose response curves that were parallel to the standard curves obtained using the kit standards.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute 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.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_Some vials contain small quantities of material, therefore centrifuge before use.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
SPARC Microplate - 96 well microplate pre-coated with a purified polyclonal anti SPARC IgG	**766-01-01**	**1 plate**
SPARC Standard – 200 ng/vial of recombinant human SPARC in a buffered protein base with preservatives; lyophilized.	**766-01-02**	**1 vial**
Biotin Solution Concentrated – 300 µL/vial, 10-fold concentrated of human SPARC biotinylated with preservatives; lyophilized.	**766-01-03**	**2 vials**
Positive Control – one vial of recombinant human SPARC , lyophilized (optional)	**766-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives. Ready to use.	**DB18**	**1 bottle**
HRP Diluent Solution- 12mL of buffered protein based solution with preservatives. Ready to use.	**DB06C**	**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 contains 0.5 M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Biotin Solution and SHOULD BE STORED at -20 °C or – 70°C for up to one month.

Reconstituted Biotin Solution (300 µl) CAN NOT BE STORED at 2-8°C. Streptavidin-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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 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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

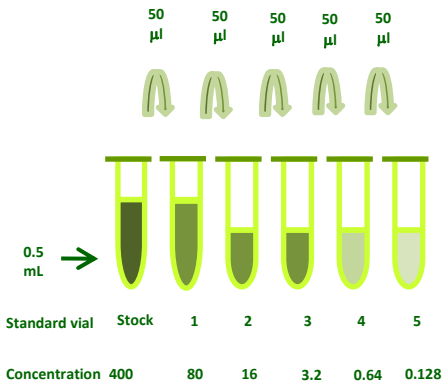
SAMPLE PREPARATION

Serum and plasma samples DO NOT require dilution. However, if the SPARC levels in samples are over 400ng/mL, a 2~4-fold or higher dilution would be required. A suggested 2-fold dilution is 60 µL sample + 60 µL Dilution Buffer. A suggested 4-fold dilution is 30 µL sample + 90 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application. Note:** PBS containing 1% BSA CAN NOT BE USED as sample matrix to dilute serum or plasma samples for this SPARC ELISA assay.
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 Wash Buffer.
Human SPARC Standard - Refer to vial label for reconstitution volume. Reconstitute the **Human SPARC** standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 400 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 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 400 ng/mL standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	0.5mL	400 ng/ml
# 1	50µl of stock	200µl	80 ng/ml
# 2	50µl of 1	200µl	16 ng/ml
# 3	50µl of 2	200µl	3.2 ng/ml
# 4	50µl of 3	200µl	0.64 ng/ml
# 5	50µl of 4	200µl	0.128 ng/ml



Biotin Solution - Reconstitute the Biotin Solution Concentrate with 300 µl of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 2.7 mL of Dilution Buffer to prepare 1x Biotin Solution.

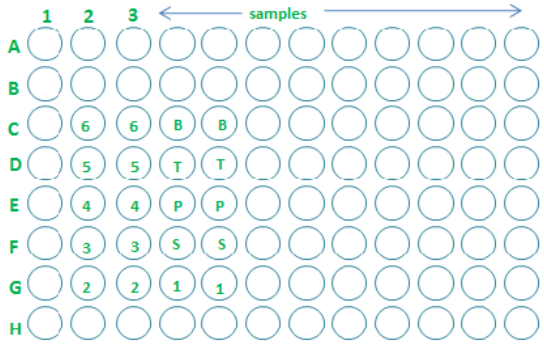
Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated Streptavidin-HRP Conjugate stock solution to 11.88 mL of **HRP Diluent Solution (DB06C)** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 0.5 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Leave well C4 and C5 as Blank. **DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.**
4. Set D4 and D5 as total binding (T). Add 50 µl per well of Dilution Buffer.
5. Add 50 µl per well of **standard solution** from #6 to S (reverse order of serial dilution) to the appropriate wells (C2, C3 to G2, G3 and F4, F5 to G4, G5). Add 50 µl per well of Positive Control into wells E4 and E5. Add 50 µl per well of samples into appropriate wells. Cover or seal the plate and incubate on microplate shaker (250-300rpm) at room temperature for 2 hours. **Note: DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.**
6. Add 50 µl per well of 1x Biotin Solution into total binding, standard, PC and samples wells. Cover or seal the plate and incubate at room temperature for 2 hours. **Note: DO NOT ADD Biotin Solution to Blank wells.**
7. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate on microplate shaker for 60 minutes at room temperature. **Protect from light**.
9. Aspirate and wash as step 7.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 18-22 minutes 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. It is recommended to add the stop solution when the Total Binding or the lowest standard has developed a dark blue color.
12. Determine the optical density of each well within 15 minutes using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and

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

CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant human SPARC.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human SPARC was 0.128 ng/mL

TYPICAL DATA

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

LAYOUT	STANDARD CONC. (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0	0 (0.095)
Stock STD	400	0.031
STD1	80	0.145
STD2	16	0.201
STD3	3.2	0.312
STD4	0.640	0.422
STD5	0.128	0.467
Total Binding	0	0.526

***Lot No.:**

****Positive Control: 34 – 65 ng/mL**

SPECIFICITY

This assay recognizes both natural and recombinant human SPARC. The factors listed below were prepared at 4000 ng/mL in Dilution Buffer, and assayed for cross reactivity.

Proteins	Cross-reactivity
Human SPARC	100%
Human Resistin	0
Human OPG	0
Human OPN	0
Human OSF-1	0
Human OGN	0
Human CTGF	0
Human Visfatin/PBEF1	0
Human Vaspin	0
Human AGTL	0

LINEARITY

To assess the linearity of the assay, samples containing human SPARC in each matrix were diluted with Dilution Buffer and then assayed.

SERUM	SPARC (NG/ML)	RECOVERY
1 x	8.536	100%
0.5 x	4.072	95.4%
0.25 x	1.716	80.4%

RECOVERY

The recovery of SPARC spiked (1:1) to human serum samples levels throughout the dynamic range of the assay was evaluated.

SPIKED SPARC (NG/ML)	RECOVERY
10	90-105%
100	85-106%

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 50 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
⬇
DO NOT ASPIRATE AND WASH PLATE. Add 50 µl 1x Biotin 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 all wells. Incubate 60 min on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 18-22 min on plate shaker. **Protect from light.**
⬇
Add 100 µl Stop Solution to each well. Read 450nm within 15 min

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