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

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

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

PURCHASE INFORMATION:

ELISA NAME	RAT/MOUSE SPARC/OSTEONECTIN ELISA
Catalog No.	SK00766-02
Lot No.	
Formulation	96 T
Standard range	0.128 - 2000 ng/mL
Dynamic range	3.2 - 2000 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	Rat/Mouse SPARC
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8°C

ORDER CONTACT:
AVISCERA BIOSCIENCE INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

INTRODUCTION

Rat/Mouse SPARC ELISA employs the quantitatively competitive enzyme immunoassay technique in which rat/mouse SPARC present in samples competed with a fixed amount of biotinylated rat/mouse SPARC for sites on an antibody specific against rat/mouse SPARC. During the incubation, the standard and samples bound to the Anti SPARC IgG which is pre-coated onto the microplates. The biotinylated SPARC are 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 Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of rat/mouse SPARC bound in the initial step. The sample values are then read off the standard curve.

Rat/Mouse SPARC ELISA has been shown to accurately quantify the recombinant and natural rat/mouse SPARC. Results obtained using natural rat/mouse 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.

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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
SPARC Microplate - 96 well microplate pre-coated with a purified antibody Anti SPARC IgG	**766-02-01**	**1 plate**
SPARC Standard – 1000 ng/vial of recombinant rat/mouse SPARC in a buffered protein base with preservatives; lyophilized.	**766-02-02**	**1 vial**
Biotin Solution Concentrate – 600 µL/vial, 10-fold concentrated of rat/mouse SPARC biotinylated with preservatives; lyophilized.	**766-02-03**	**1 vial**
Positive Control – one vial of recombinant rat/mouse SPARC , lyophilized (optional)	**766-02-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservatives. Ready to use.	**DB18**	**1 bottle**
HRP Diluent Solution – 12 mL 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 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 6 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Biotin Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Reconstituted Biotin Solution (600 µl) CANNOT BE STORED at 2-8°C. Streptavidin-HRP Conjugate 200-fold Concentrate and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

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.

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 2000 ng/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 CANNOT BE USED as sample matrix to dilute serum or plasma samples for this SPARC ELISA assay.
Use polypropylene test tubes.

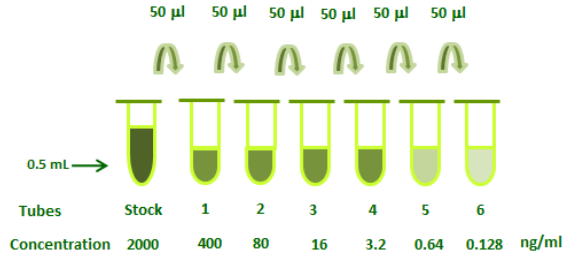
REAGENT PREPARATION

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.

Rat/Mouse SPARC Standard - Refer to vial label for reconstitution volume. Reconstitute the **Rat/Mouse SPARC** standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 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 2000 ng/mL standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	0.5 mL	2000 ng/ml
# 1	50µl of stock	200 µl	400 ng/ml
# 2	50µl of 1	200 µl	80 ng/ml
# 3	50µl of 2	200 µl	16 ng/ml
# 4	50µl of 3	200 µl	3.2 ng/ml
# 5	50µl of 4	200 µl	0.64 ng/ml
# 6	50ul of 5	200 µl	0.128 ng/ml



Biotin Solution Concentrate - Reconstitute the Biotin Solution Concentrate with 600 µL of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 5.4 mL of Dilution Buffer in a 15 mL centrifuge tube to prepare **1X Biotin Solution**.

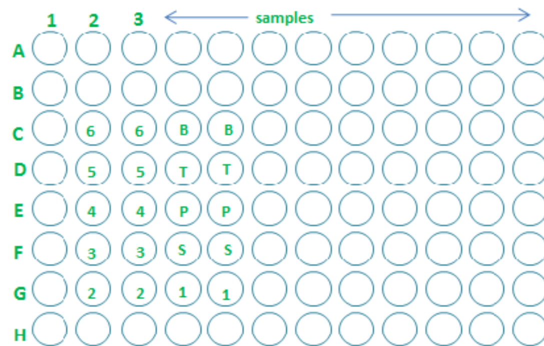
Streptavidin-HRP Conjugate - Transfer 60 µL of 200-fold concentrated Streptavidin-HRP Conjugate stock solution to 11.94 mL of **HRP Diluent Solution** to prepare working solution. **Note:** 1X working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used within a few days.

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 and seal.
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 micro-plate 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 micro-plate 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 15 - 90 seconds at room temperature. **Protect from light**. **Note: Please pay careful attention due to the quick development of color.**
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 rat/mouse SPARC.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of rat/mouse 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 (0.039)
Stock STD	2000	0.030
STD1	400	0.125
STD2	80	0.326
STD3	16	0.566
STD4	3.2	0.770
STD5	0.640	0.863
STD6	0.128	0.899
Total Binding	0	0.852

- **Lot No.:**
- **Positive Control: 7.0 – 15 ng/mL**

LINEARITY

To assess the linearity of the assay, research **mouse plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1x	15.054	15.054	100
2.5x	7.135	17.8375	118
5x	4.226	21.13	140

To assess the linearity of the assay, research **mouse serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1x	24.466	24.466	100
5x	6.308	31.54	129

To assess the linearity of the assay, research **rat plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1x	24.764	24.764	100
5x	5.083	25.415	103

To assess the linearity of the assay, research **rat serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1x	34.555	34.555	100
2.5x	11.129	27.8225	80.5
5x	5.829	29.145	84.3

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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 15-90 sec on plate shaker at RT. **Protect from light.**
↓
Add 100 μl Stop Solution to each well. Read 450nm within 15 min