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

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.

PRODUCT 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	0.64 - 2000 ng/mL
Sensitivity	1 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
Calibration	Rat/Mouse SPARC Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
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

Rat/Mouse SPARC ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural rat/mouse SPARC from serum and plasma in a competitive enzyme immunoassay technique format.

This immunoassay contains recombinant rat/mouse SPARC and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural rat/mouse SPARC samples.

ASSAY OVERVIEW

Rat/Mouse SPARC ELISA employs the quantitatively competitive enzyme immunoassay technique in which rat/mouse SPARC present in samples compete 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 bind to the anti-SPARC IgG pre-coated onto the microplate. The biotinylated SPARC competitively bind to the 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.

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
SPARC Microplate - 96 well microplate pre-coated with a purified antibody anti-SPARC IgG.	**766-02-01**	**1 plate**
SPARC Standard – 2000 ng/vial of recombinant rat/mouse SPARC in a buffered protein base with preservative; lyophilized.	**766-02-02**	**1 vial**
Biotin Solution Concentrate – 600 µL/vial of 10-fold concentrate of rat/mouse SPARC biotinylated with preservative; 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 - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative. Ready to use.	**DB18**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**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 (stock) solution and Biotin concentrated 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 100-fold concentrated solution **(DO NOT FREEZE** and **PROTECT FROM LIGHT**) 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. Microplate may be stored for up to 6 months at 2 – 8° C.

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 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

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.

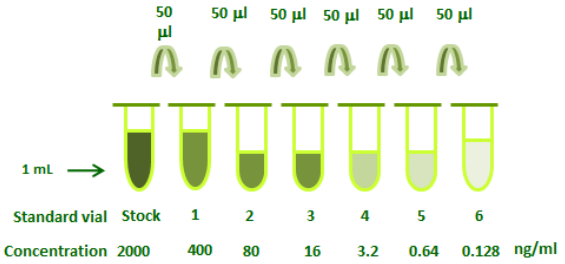
REAGENT PREPARATION

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 1x Wash Buffer.

Rat/Mouse SPARC Standard - Reconstitute the rat/mouse SPARC standard with 1.0 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	1.0 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



Positive Control - Reconstitute the Positive Control with 2.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C or -70° C.

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 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 **(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. Leave two wells as Blank. **DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.**
4. Set two wells as total binding. Add 50 µl per well of Dilution Buffer.
5. Add 50 µl per well of **Standard dilutions** from #6 to #S (reverse order of serial dilution) to the appropriate wells. Add 50 µl per well of **Positive Control** into wells. Add 50 µl per well of **samples** into appropriate wells. Cover with plate sealer and incubate on microplate shaker (250-300 rpm) 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, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker 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 45 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 4-7 minutes on microplate shaker 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 microplate 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.

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TYPICAL DATA

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

LAYOUT	STANDARD CONC. (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank		0.064
Stock STD	2000	0.014
STD1	400	0.160
STD2	80	0.358
STD3	16	0.703
STD4	3.2	0.878
STD5	0.640	1.057
STD6	0.128	1.062
Total Binding	0	1.084

- **Lot No.:**
- **Positive Control:**

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇓
Add 50 µl of standard, samples, positive control to the well. Incubate 2 hours on the plate shaker at RT.
⇓
DO NOT ASPIRATE AND WASH PLATE. Add 50 µl 1x Biotin Solution to the 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 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 4-7 minutes on plate shaker at RT. **Protect from light**.
⇓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.