OriGene Technologies Inc.
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Human SPARC ELISA Kit

Catalog Number: EA100715

Assay Principle

The OriGene Human SPARC Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human SPARC with a 96-well strip plate that is pre-coated with antibody specific for SPARC. The detection antibody is a biotinylated antibody specific for SPARC. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human SPARC with immunogen: Expression system for standard: NSO; Immunogen sequence: A18-I303. The kit is analytically validated with ready to use reagents.

To measure Human SPARC, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Human SPARC in the sample. Read the density of the yellow product in each well using a plate reader and benchmark the sample wells' readings against the standard curve to determine the concentration of Human SPARC in the sample.

Overview

Product Name	Human SPARC ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human SPARC. 96wells/kit, with removable strips.
Sensitivity	<20 pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	0.78 ng/ml - 50 ng/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
Uniprot ID	P09486

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Technical Details

Capture/Detection Antibodies	*The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.*
Specificity	*Natural and recombinant Human SPARC*
Immunogen	*Expression system for standard: NSO; Immunogen sequence: A18 – I303*
Cross Reactivity	*There is no detectable cross-reactivity with other relevant proteins.*

Notice Before Application

Please read the following instructions before starting the experiment.

1. *To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.*
2. *Before using the Kit, spin tubes and bring down all components to the bottom of tubes.*
3. *Don't let 96-well plate dry, for dry plate will inactivate active components on plate.*
4. *Don't reuse tips and tubes to avoid cross contamination.*
5. *Avoid using the reagents from different batches together.*

Kit Components/Materials Provided

Description	Quantity	Volume	Storage of opened/reconstituted material
Anti-Human SPARC Pre-coated 96-well strip microplate	**1**	**12 strips of 8 wells**	**Return unused wells to the foil pouch. Reseal along the entire edge of the zip seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Human SPARC Standard	**2**	**50 ng/tube**	**Discard the SPARC stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.**
Human SPARC Biotinylated antibody (100x)	**1**	**100 µl**	**May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Avidin-Biotin-Peroxidase Complex (100x)	**1**	**100 µl**	
Sample Diluent	**1**	**30ml**	
Antibody Diluent	**1**	**12ml**	
Avidin-Biotin-Peroxidase Diluent	**1**	**12ml**	
Color Developing Reagent (TMB)	**1**	**10ml**	
Stop Solution	**1**	**10ml**	
Wash Buffer (25x)	**1**	**20 ml**	
Plate Sealers	**4**	**Piece**	

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Required Materials That Are Not Supplied

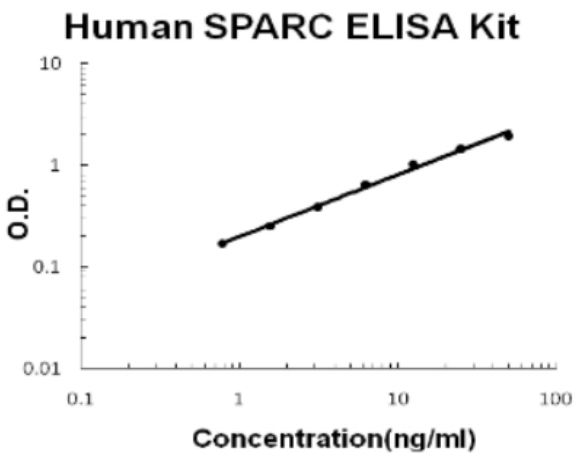
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Human IL-1 Beta ELISA Kit (EA100715) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	0.78	1.56	3.12	6.25	12.5	25	50
O.D.	0.099	0.169	0.251	0.388	0.638	1.020	1.466	1.931

Human Serpin A1 ELISA Kit standard curve



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

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): *Three samples of known concentration were tested on one plate to assess intra-assay precision.*
Inter-Assay Precision (Precision across assays): *Three samples of known concentration were tested in separate assays to assess inter-assay precision.*

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	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	1247	5311	21553	1248	5208	22753
Standard deviation	93.52	393.01	1012.99	99.84	457.86	1296.92
CV(%)	7.5	7.4	4.7	8	8.8	5.7

Reproducibility

To assay reproducibility, three samples with different target protein concentrations were assayed using four different lots. Number of each test n = 16.

Lots	Lot 1(pg/ml	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviations	CV (%)
Sample1	1247	1275	1224	1339	1271	43.08	3.3 %
Sample2	5311	5517	5748	5864	5610	213.07	3.7 %
Sample3	21553	20541	21138	20396	20907	465.22	2.2 %

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C.
Wash buffer	Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-human SPARC antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human SPARC Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human SPARC Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 50 ng of lyophilized Human SPARC standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 50 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

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Dilution of Human SPARC Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –50,000 pg/ml, #2 –25,000 pg/ml, #3 –12,500 pg/ml, #4 –6,250 pg/ml, #5 –3,1250 pg/ml, #6 – 1562.50 pg/ml, #7 – 781.25 pg/ml, #8 – 0.0 (Blank).
2. For standard #1, add 1000 µl of undiluted standard stock solution of 50 ng/ml to tube #1 for a final volume of 1000 µl.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Preparation andStorage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Milk	Centrifuge for 15 min at 1500 x g at 2-8°C. Collect the aqueous layer and repeat this process 3 times. Filter through a 0.2 µm filter. Assay immediately or aliquot and store samples at -20°C.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.
It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.

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4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Human SPARC antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
   d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 4 additional times.
    d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data
For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on SPARC

SPARC (secreted protein acidic and rich in cysteine), also known as Osteonectin, is a protein that in humans is encoded by the SPARC gene. The human SPARC gene is 26.5 kb long, and contains 10 exons and 9 introns and is located on chromosome 5q31-q33. SPARC is a glycoprotein of 40 kD. SPARC is an acidic, cysteine-rich glycoprotein consisting of a single polypeptide chain that can be broken into 4 domains: 1) an Ca++ binding domains near the glutamic acidic-rich region at the amino terminus (domain I), 2) a cysteine- rich (domain II), 3) a hydrophilic region (domain III) and 4) an EF hand motif at the carboxy terminus region (domain IV). Osteonectin is a glycoprotein in the bone that binds sodium. It is secreted by osteoblasts during bone formation, initiating mineralization and promoting mineral crystal formation. Osteonectin also shows affinity for collagen in addition to bone mineral calcium. A correlation between osteonectin over expression and ampullary cancers and chronic pancreatitis has been found.