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ab213826 – Human SPARCL1 ELISA Kit

For the quantitative detection of Human SPARCL1 in cell culture supernatants and serum.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

The Human SPARCL1 Enzyme-Linked Immunosorbent Assay (ELISA) kit (ab213826) is designed for the quantitative measurement of Human SPARCL1 in cell culture supernatants and serum.

The ELISA kit is based on standard sandwich enzyme-linked immunosorbent assay technology. A monoclonal antibody from mouse specific for SPARCL1 has been pre-coated onto 96-well plates. Standards (Expression system for standard: NSO; Immunogen sequence: I17-F66) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for SPARCL1 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic TMB Stop Solution. The density of yellow is proportional to the Human SPARCL1 amount of sample captured in plate.

SPARCL1 (SPARC-Like Protein 1), also known as HEVIN, is a protein that in humans is encoded by the SPARCL1 gene. The cells in high endothelial venules (HEVs) in lymphoid tissues have a plump, almost cuboidal, appearance and support high levels of lymphocyte extravasation from blood, possibly due to the presence of desmosome-like junctions rather than tight junctions in the HEVs. In chronic inflammation, the activated endothelium of non-lymphoid tissues acquires an HEV-like morphology and function. SPARCL1 is highly expressed in HEV and is thought to contribute to the induction or maintenance of features of the HEV endothelium that facilitate lymphocyte migration.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 100 µL standard or sample to appropriate wells

Incubate at 37°C for 90 minutes

Discard plate content. Do not wash.

Add 100 µL biotinylated Antibody in to all wells

Incubate at 37°C for 60 minutes

Wash each well three times with 300 µL 0.01M PBS (or TBS)

Add 100 µL ABC working solution

Incubate at 37°C for 30 minutes

Wash each well five times with 300 µL 0.01M PBS (or TBS)

Add 90 µL of prepared TMB

Incubate at 37°C in dark for 15-20 minutes

Add 100 µL TMB Stop Solution and read OD at 450 nm within 30 minutes

3. Precautions

Please read these instructions carefully prior to beginning the ELISA assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store ELISA kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
Anti-Human SPARCL1 coated Microplate (12 x 8 wells)	1 x 96 well plate	-20°C	-20°C
Lyophilized recombinant Human SPARCL1 standard	2 x 1 vial	-20°C	-20°C
Biotinylated anti-Human SPARCL1 antibody	130 µL	-20°C	-20°C
Avidin-Biotin-Peroxidase Complex (ABC)	130 µL	-20°C	-20°C
Sample diluent buffer	30 mL	-20°C	-20°C
Antibody diluent buffer	12 mL	-20°C	-20°C
ABC diluent buffer	12 mL	-20°C	-20°C
TMB	10 mL	-20°C	-20°C
TMB Stop Solution	10 mL	-20°C	-20°C
Adhesive Plate Seal	4	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Automated plate washer.
- Multi- and single-channel pipettes.
- Clean tubes and Eppendorf tubes.
- Washing buffer (neutral 0.01M PBS or 0.01M TBS).
  - Preparation of 0.01M TBS: Add 1.2 g Tris, 8.5 g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to 1,000 mL distilled water and adjust pH to 7.2~7.6. Finally, adjust the total volume to 1 L.
  - Preparation of 0.01M PBS: Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1,000 mL distilled water and adjust pH to 7.2~7.6. Finally, adjust the total volume to 1 L.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Don't let the 96-well plate dry, for a dry plate will inactivate active components on plate.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 minutes before using.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Anti-Human SPARCL1 coated Microplate (12 x 8 wells)

One plate of 96 wells. Ready to use. Store at -20°C.

9.2 Lyophilized recombinant Human SPARCL1 standard (2 x 10 ng)

9.2.1 SPARCL1 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of SPARCL1 standard (2 x 10 ng) are included in each kit. Use one tube for each experiment.

9.2.2 Add 1 mL sample diluent buffer into one tube to create 10,000 pg/mL of Human SPARCL1 stock solution. Keep the tube at room temperature for 10 minutes and mix thoroughly.

9.3 Biotinylated anti-Human SPARCL1 antibody

The solution should be prepared no more than 2 hours prior to the experiment.

9.3.1 The total volume should be: 100 µL/well x (the number of wells). (Allowing 100 µL – 200 µL more than total volume)

9.3.2 Biotinylated anti-Human SPARCL1 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated anti-Human SPARCL1 antibody to 99 µL antibody diluent buffer.)

9.4 Avidin-Biotin-Peroxidase Complex (ABC)

The solution should be prepared no more than 1 hour prior to the experiment.

9.4.1 The total volume should be: 100 µL/well x (the number of wells). (Allowing 100 µL - 200 µL more than total volume)

9.4.2 Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1 µL ABC to 99 µL ABC diluent buffer.)

9.5 Sample diluent buffer

30 mL. Ready to use. Store at -20°C.

9.6 Antibody diluent buffer

12 mL. Ready to use. Store at -20°C.

9.7 ABC diluent buffer

12 mL. Ready to use. Store at -20°C.

9.8 TMB

10 mL. Ready to use. Store at -20°C.

9.9 TMB Stop Solution

10 mL. Ready to use. Store at -20°C.

10. Standard Preparation

10.1 Prepare a 3,000 pg/mL SPARCL1 solution by adding 300 µL of the above 10,000 pg/mL SPARCL1 stock solution into a tube with 700 µL sample diluent buffer and mix thoroughly.

10.2 To prepare standards, label 6 Eppendorf tubes with 1,500 pg/mL, 750 pg/mL, 375 pg/mL, 187.5 pg/mL, 93.75 pg/mL and 46.875 pg/mL respectively.

10.3 Aliquot 300 µL of the sample diluent buffer into each tube.

10.4 Add 300 µL of the above 3,000 pg/mL SPARCL1 solution into 1st tube and mix.

10.5 Transfer 300 µL from 1st tube to 2nd tube and mix. Transfer 300 µL from 2nd tube to 3rd tube and mix, and so on.

Tube #	Volume to dilute	Volume of diluent	Concentration (pg/mL)
1	300 µL of 10,000 pg/mL stock solution	700 µL	3,000
2	300 µL of 3,000 pg/mL stock solution	300 µL	1,500
3	300 µL of tube #2	300 µL	750
4	300 µL of tube #3	300 µL	375
5	300 µL of tube #4	300 µL	187.5
6	300 µL of tube #5	300 µL	93.75
7	300 µL of tube #6	300 µL	46.875

Δ Note: The standard solutions are best used within 2 hours. The 10,000 pg/mL standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

11. Sample Preparation

Store samples to be assayed within 24 hours at 4°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.
- Cell culture supernatant: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

It is recommended to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- High target protein concentration (30,000 pg/mL-300,000 pg/mL). The working dilution is 1:100. i.e. Add 1 µL sample into 99 µL sample diluent buffer.
- Medium target protein concentration (3,000 pg/mL-30,000 pg/mL). The working dilution is 1:10. i.e. Add 10 µL sample into 90 µL sample diluent buffer.
- Low target protein concentration (46.9 pg/mL-3,000 pg/mL). The working dilution is 1:2. i.e. Add 50 µL sample to 50 µL sample diluent buffer.
- Very Low target protein concentration (0 pg/mL-46.9 pg/mL). No dilution necessary, or the working dilution is 1:2.

12. Assay Procedure

- It is recommended to assay all standards, controls and samples in duplicate.
- The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard SPARCL1 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of SPARCL1 amount in samples.

12.1 Aliquot 100 µL per well of the 3,000 pg/mL, 1,500 pg/mL, 750 pg/mL, 375 pg/mL, 187.5 pg/mL, 93.75 pg/mL and 46.875 pg/mL Human SPARCL1 standard solutions into the pre-coated 96-well plate.

12.2 Add 100 µL of the sample diluent buffer into the control well (Zero well).

12.3 Add 100 µL of each properly diluted sample of Human cell culture supernatants or serum to each empty well. See "Sample Preparation" above for details. It is recommended that each Human SPARCL1 standard solution and each sample be measured in duplicate.

12.4 Seal the plate with a new adhesive cover provided and incubate at 37°C for 90 minutes.

12.5 Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

12.6 Add 100 µL of biotinylated anti-Human SPARCL1 antibody working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C for 60 minutes.

12.7 Wash plate 3 times with 0.01M TBS or 0.01M PBS and each time let washing buffer stay in the wells for 1 minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 300 µL PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of three washes. Note: For automated washing, aspirate all wells and wash three times with PBS or TBS buffer, overfilling wells with PBS

or TBS buffer. Blot the plate onto paper towels or other absorbent material.)

12.8 Add 100 µL of prepared ABC working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C for 30 minutes.

12.9 Wash plate 5 times with 0.01M TBS or 0.01M PBS and each time let washing buffer stay in the wells for 1-2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 12.7 for plate washing method.)

12.10 Add 90 µL of prepared TMB color developing agent into each well, seal the plate with a new adhesive cover and incubate at 37°C in dark for 15-20 minutes.

Δ Note: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated Human SPARCL1 standard solutions; the other wells show no obvious color.

12.11 Add 100 µL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.

12.12 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the TMB Stop Solution.

13. Calculations

The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human SPARCL1 concentration of the samples can be interpolated from the standard curve.

(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well).

Δ Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

14. Typical data

Typical standard curve – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Sample	Human SPARCL1 (pg/mL)	O.D.
1	0	0.075
2	46.9	0.102
3	94	0.142
4	188	0.222
5	375	0.382
6	750	0.728
7	1,500	1.383
8	3,000	2.301

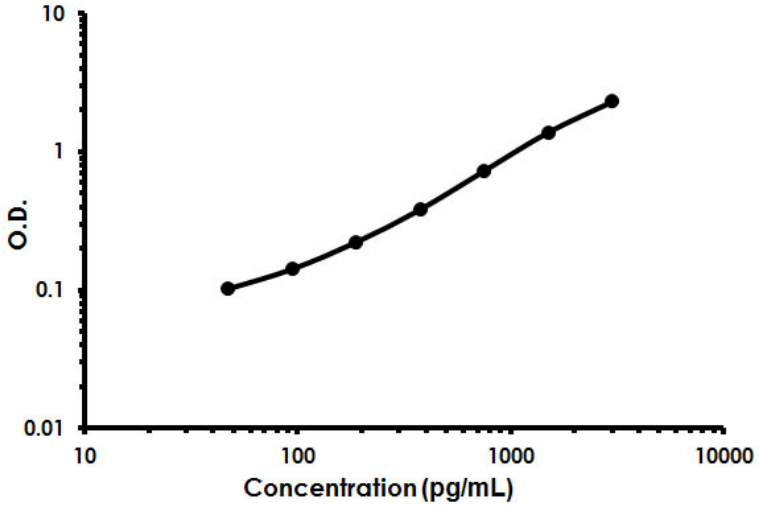

Figure 1. Human SPARCL1 ELISA Kit (ab213826) Standard Curve.

15. Typical sample values

Sensitivity –

The biological sensitivity of the assay is <10 pg/mL.

The range is 46.9 pg/mL – 3,000 pg/mL.

Precision –

Intra-assay precision: (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Sample	Number of measures	Mean (pg/mL)	Standard Deviation	CV%
1	16	269	11.03	4.1
2	16	1,195	57.36	4.8
3	16	2,030	105.60	5.2

Inter-assay precision: (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sample	Number of assays	Mean (pg/mL)	Standard Deviation	CV%
1	24	321	18.3	5.7
2	24	1,254	79.0	6.3
3	24	2,208	167.8	7.6

Specificity:

Natural and recombinant Human SPARCL1.

Cross-reactivity:

There is no detectable cross-reactivity with other relevant proteins.

16. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check Pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check Pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of TMB Stop Solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All components 4°C. Keep TMB substrate solution protected from light.

17. Notes

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