Product Manual

Scavenger Receptor Class B Member 1 (SRB1) ELISA Kit

Catalog Number

STA-630 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Cholesterol is an essential component of cellular membranes, as well as an essential substrate for steroid hormones and bile acid synthesis. However, cholesterol is toxic when accumulated in excess in cellular membranes, and elaborate pathways have evolved to control its synthesis, uptake and storage. Cells can obtain cholesterol either from de novo synthesis or uptake from circulating lipoproteins. While the low-density lipoprotein receptor (LDLR) is the primary pathway for removal of cholesterol from circulation, cholesterol within high density lipoprotein (HDL) is also removed from circulation through binding and endocytosis of the Scavenger Receptor Class B Member 1 (SRB1).

SRB1 is a tetrameric transmembrane protein of 509 amino acids. SRB1 plays a critical role in the reverse cholesterol transport pathway (Figure 1), where cholesterol from macrophages and peripheral tissues is cleared and transported to the liver. While SRB1 has been shown to mediate binding of HDL and selective uptake of cholesteryl esters (CEs), the mechanism of CE uptake is not entirely clear. Unlike LDLR, which mediates endocytosis of the entire LDL particle into vesicles, the prevailing view for SRB1 is that it mediates HDL-CE transfer from the hydrophobic core of HDL without the internalization and subsequent degradation of the lipoprotein particle. In addition to its role in movement of CE, SRB1 overexpression has been shown to alter plasma membrane composition itself, increasing the levels of cholesterol within the plasma membrane.

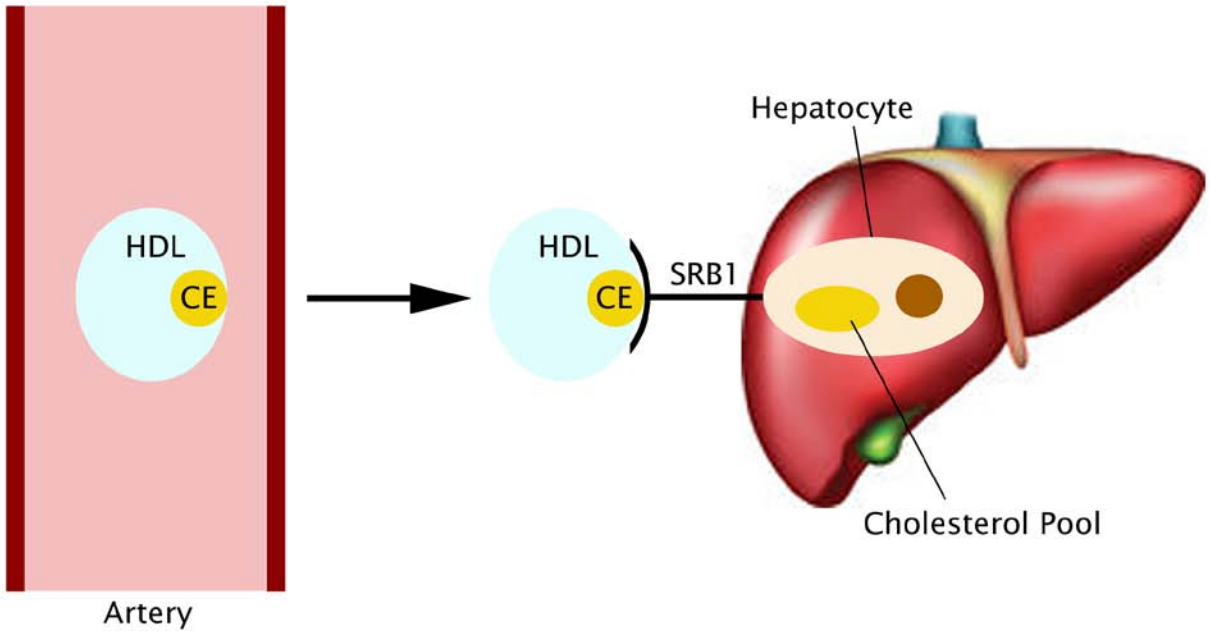

Figure 1. The HDL cholesterol transport pathway.

Cell Biolabs' Scavenger Receptor Class B Member 1 (SRB1) ELISA Kit is an enzyme immunoassay for the detection and quantitation of human, mouse, rat, or hamster SRB1 in plasma, serum, cell or tissue lysate samples. The kit has a detection sensitivity limit of 600 pg/mL human SRB1. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
2. STA-368: Human ApoB ELISA Kit
3. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-387: Human LOX-1 ELISA Kit
5. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
6. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)
7. STA-386: Human LDLR ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-SRB1 Antibody Coated Plate (Part No. 263001): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-SRB1 Antibody (1000X) (Part No. 263002): One 12 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human SRB1 Standard (Part No. 263003): One 20 µL vial of 10 µg/mL human recombinant SRB1 (amino acids 32-440) in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum, Cell or Tissue Lysate
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human SRB1 Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-SRB1 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Human SRB1 antibody and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human SRB1 Standard

Prepare a dilution series of human SRB1 standards in the concentration range of 0 to 20 ng/mL in Assay Diluent (Table 1).

Standard Tubes	10 µg/mL Human SRB1 Standard (µL)	Assay Diluent (µL)	Human SRB1 (ng/mL)
1	2	998	20
2	400 of Tube #1	400	10
3	400 of Tube #2	400	5
4	400 of Tube #3	400	2.5
5	400 of Tube #4	400	1.25
6	400 of Tube #5	400	0.625
7	400 of Tube #6	400	0.3125
8	0	400	0

Table 1. Preparation of Human SRB1 Standards

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold RIPA buffer and centrifuge at 14,000 x g for 15 minutes at 4ºC. Isolate the supernatant and assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of SRB1 unknown sample or standard to the Anti-SRB1 Antibody Coated Plate. Each SRB1 unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-SRB1 Antibody to each well. Incubate at room temperature for 2 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the SRB1 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

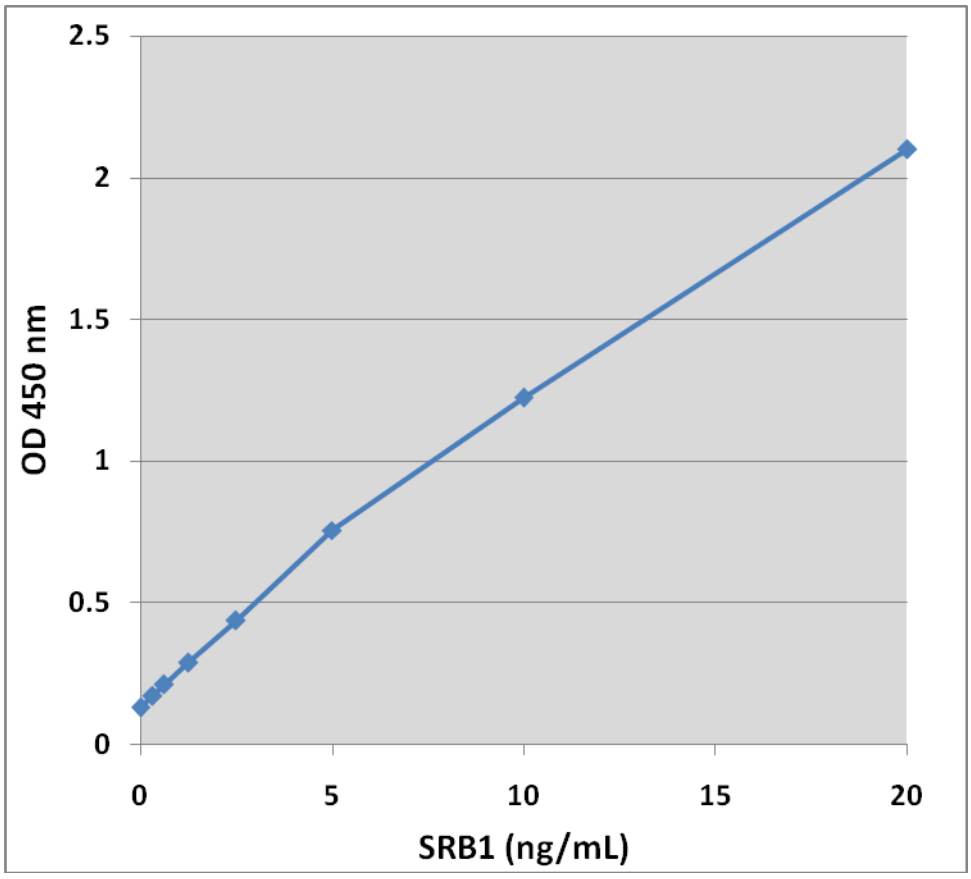

Figure 2: Human SRB1 ELISA Standard Curve.

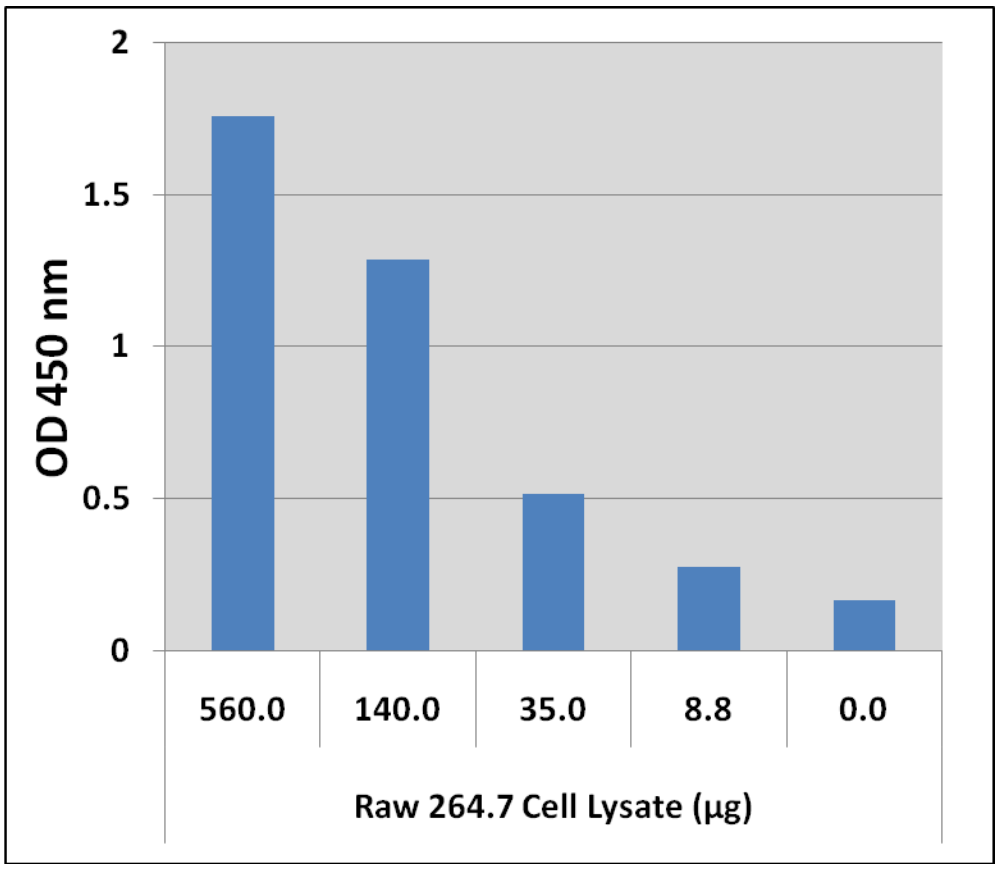

Figure 3. Detection of SRB1 in mouse Raw 264.7 Cells. Raw Cells were washed with PBS, lysed with RIPA buffer, and centrifuged for 15 minutes at 20000 xg. The supernatant was recovered and added to the wells according to the SRB1 ELISA kit protocol.

References

1. Brown M. S., and Goldstein J. L. (1986). *Science* **232**, 34-47.
2. Brown M. S., and Goldstein J. L. (1997). *Cell* **89**, 331-340.
3. Han C-H. and Lee M-H. (2002). *J. Vet. Sci.* **3**:265-272.
4. De la Llera-Moya M., Rothblat G.H., Connelly M.A., Kellner-Weibel G., Sakr S.W., Phillips M.C., and Willisma D.L. (1999) *J. Lipid Res* **40**:575-580
5. Kent A.P. and Stylianou I.M. (2011) *Hep. Med. Evid. Res*. **3**:29-44.

Warranty

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