

REFERENCES

1. Prakobphol A, et al. (1998). *Biochemistry*. 37 (14): 4916–27.
2. Kohn LA, et al. (2012). *Nat. Immunol.* 13 (10): 963–71.
3. Brenner B, et al. (1997). *Proc. Natl. Acad. Sci. U.S.A.* 93 (26): 15376–81.
4. Zöllner O, et al. (1997). *J. Cell Biol.* 136 (3): 707–16.
5. Sassetti C, et al. (1998). *J. Exp. Med.* 187 (12): 1965–75.
6. Malhotra R, et al. (1999). *Biochem. J.* 341 (1): 61–9.
7. Goldson TM, et al. (2020) *Am. J. Physiol., Cell Physiol.* 318 (1), C83–C93.
8. Lokwani R, et al. (2019) *Int J Chron Obstruct Pulmon Dis* 14, 2517-2525.
9. Bar-Ephraim YE, et al. (2019) *J. Immunol.* 202 (1), 171-182.
10. Kononchik J, et al. (2018) *Nat Commun* 9 (1), 2825.

Human L-selectin Immunoassay

Catalog Number:SEKH-0231

For the quantitative determination of human L-selectin concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

Country | Company: China | Beijing Solarbio Science & Technology Co.,Ltd
Address:NO.85A, Liandong U Valley, Tongzhou District, Beijing, P.R.China.
Tel: 86-10-56371241 Fax: 86-10-56371282 E-mail: service@solarbio.com

TABLE OF CONTENTS

| SECTION | PAGE |
|---|------|
| BACKGROUND..... | 01 |
| PRINCIPLE OF THE ASSAY..... | 01 |
| TECHNICAL HINTS AND LIMITATIONS..... | 02 |
| PRECAUTIONS..... | 02 |
| KIT COMPONENTS& STORAGE CONDITIONS..... | 03 |
| OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED..... | 04 |
| SPECIMEN COLLECTION & STORAGE..... | 04 |
| REAGENTS PREPARATION..... | 04 |
| ASSAY PROCEDURE..... | 06 |
| CALCULATION OF RESULTS..... | 06 |
| PERFORMANCE CHARACTERISTICS..... | 08 |
| REFERENCES..... | 10 |

LINEARITY:To assess the linearity of the assay, three samples were spiked with high concentrations of L-selectin in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

| Dilution ratio | Recovery(%) | Citrate plasma | Cell culture supernatants |
|----------------|----------------------|----------------|---------------------------|
| 1:2 | Average% of Expected | 93 | 104 |
| | Range(%) | 85-107 | 95-111 |
| 1:4 | Average% of Expected | 94 | 104 |
| | Range(%) | 85-104 | 101-117 |
| 1:8 | Average% of Expected | 98 | 99 |
| | Range(%) | 92-109 | 88-110 |
| 1:16 | Average% of Expected | 94 | 105 |
| | Range(%) | 90-103 | 96-112 |

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 0.15ng/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human L-selectin. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

| Recombinant human | Recombinant mouse | Recombinant porcine |
|-------------------|-------------------|---------------------|
| ApoAI | | |
| BMP1 | | |
| CRP | | |
| HGF | | |
| HSP27 | | |
| IL-1 α | | |
| MMP-2 | | |

REPEATABILITY:The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of L-selectin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of L-selectin in two matrices

| Sample Type | Average % of Expected Range(%) | Range(%) |
|---------------------------|--------------------------------|----------|
| Citrate plasma | 93 | 87-101 |
| Cell culture supernatants | 106 | 97-112 |

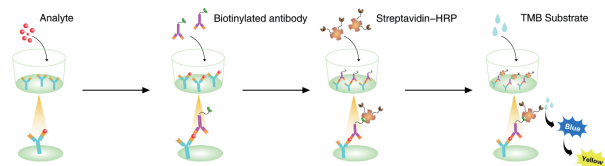
BACKGROUND

L-selectin (SELL), also known as CD62L, is a cell adhesion molecule found on leukocytes and the preimplantation embryo. SELL is a cell surface component that is a member of a family of adhesion/homing receptors that play important roles in lymphocyte-endothelial cell interactions. L-selectin acts as a "homing receptor" for lymphocytes to enter secondary lymphoid tissues via high endothelial venules. Ligands present on endothelial cells will bind to lymphocytes expressing L-selectin, slowing lymphocyte trafficking through the blood, and facilitating entry into a secondary lymphoid organ at that point. Central memory T-lymphocytes, which have encountered antigen, express L-selectin to localize in secondary lymphoid organs. High expression of L-selectin on human bone marrow progenitor cells is an early sign of cells becoming committed to lymphoid differentiation. L-selectin acts as a receptor to facilitate adhesion of the embryo to the site of invasion on the surface epithelium of the uterine endometrium. Removal of MUC-1 exposes the oligosaccharide ligands of the uterine epithelium, thus allowing binding by the L-selectin receptor of the trophoblast cell, followed by embryo adhesion and invasion.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for L-selectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any L-selectin present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for L-selectin is added to detect the captured L-selectin protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

DESCRIPTION



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

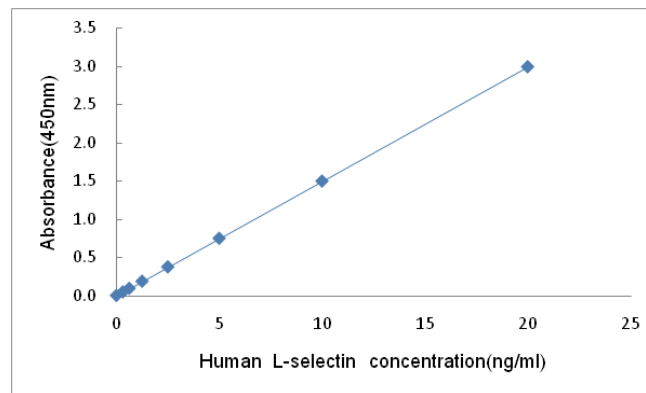
DESCRIPTION

determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by 5. the dilution factor.

This standard curve is provided for demonstration only. A standard

Typical data using the L-selectin ELISA

| Standardized (ng/ml) | OD. | OD. | Average | Corrected |
|----------------------|-------|-------|---------|-----------|
| 0 | 0.048 | 0.048 | 0.048 | -- |
| 0.31 | 0.188 | 0.177 | 0.183 | 0.134 |
| 0.6 | 0.273 | 0.258 | 0.266 | 0.217 |
| 1 | 0.406 | 0.383 | 0.395 | 0.347 |
| 2.5 | 0.645 | 0.609 | 0.627 | 0.579 |
| 5 | 1.048 | 0.989 | 1.019 | 0.970 |
| 10 | 1.707 | 1.610 | 1.658 | 1.610 |
| 20 | 2.781 | 2.623 | 2.702 | 2.654 |



Representative standard curve for L-selectin ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash three times before assay.



Add 100µl standard or samples to each well, incubate 120 minutes, at room temperature (25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human L-selectin antibody to each well, incubate 60 minutes, at room temperature (25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes, at room temperature (25±2°C).



Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 30 minutes, at room temperature (25±2°C). Protect from light.



Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

Note: oscillatory reaction at room temperature 400r

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the L-selectin concentrations versus the log of the O.D. and the best fit line can be

KIT COMPONENTS & STORAGE CONDITIONS

| PART | SIZE | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|--|----------|---|
| Microwell Plate - antibody coated 96-well Microplate (8 wells x 12 strips) | 1 plate | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C** |
| Standard - lyophilized, 20 ng/ml upon reconstitution | 2 vials | Aliquot and Store at -20°C** for six months |
| Concentrated Biotin-Conjugated antibody (100X) - 120 ul/vial | 1 vial | Store at 2-8°C ***for six months |
| Concentrated Streptavidin-HRP solution (100X) - 120 ul/vial | 1 vial | Store at 2-8°C ***for six months |
| Standard /sample Diluent - 16 ml/vial | 1 bottle | Store at 2-8°C ***for six months |
| Biotin-Conjugate antibody Diluent - 16 ml/vial | 1 bottle | Store at 2-8°C ***for six months |
| Streptavidin-HRP Diluent - 16 ml/vial | 1 bottle | Store at 2-8°C ***for six months |
| Wash Buffer Concentrate (20x) - 30 ml/vial | 1 bottle | Store at 2-8°C ***for six months |
| Substrate Solution - 12 ml/vial | 1 bottle | Store at 2-8°C ***for six months |
| Stop Solution - 12 ml/vial | 1 bottle | Store at 2-8°C ***for six months |
| Plate Cover Seals | 4 pieces | |

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirrt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge approximately for 15 minutes at 1000g (or 3000rpm). Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g (or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25°C) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

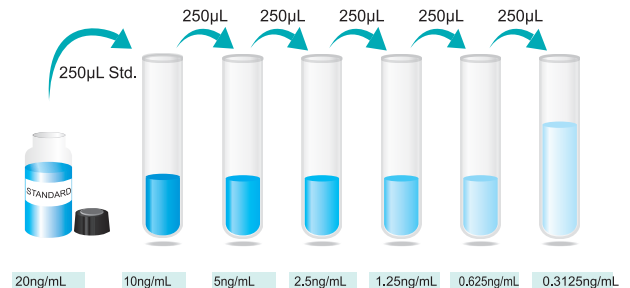
3. **Standard/Sample - Reconstitute the Standard with 0.5mL of Standard/Sample Diluent.** This reconstitution produces a stock solution of 20ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 μL of Standard/Sample Diluent into 10ng/ml tube and the remaining tubes. Use the stock solution of 20ng/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 20ng/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).
*If you do not run out of re-melting standard, store it at -20°C . Diluted standard shall not be reused.

4. **Working solution of Biotin-Conjugate anti-human L-selectin antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate antibody with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

*The working solution should be used within one day after dilution.

5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

*The working solution should be used within one day after dilution.



Preparation of L-selectin standard dilutions