Human sE-selectin ELISA Kit

Catalog Number
EK0491

For the quantitative determination of human soluble E-Selectin (sE-Selectin) concentrations in cell culture supernates, serum, and plasma.

This instruction must be read in its entirety before using this product.

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

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sE-selectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sE-selectin present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for sE-selectin is added to the wells and binds to the combination of capture antibody- sE-selectin in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of sE-selectin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven sE-selectin standard dilutions and sE-selectin sample concentration determined.

DETECTION RANGE

780 pg/ml - 50000 pg/ml

SENSITIVITY

The minimum detectable dose was 200pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human sE-selectin. The factors listed below were prepared at 160ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 1: Factors assayed for cross-reactivity

Recombinant human	**Recombinant mouse**	**Recombinant rat**
ICAM-1	E-Selectin	E-Selectin
ICAM-2	Calectin-1	ICAM-1
ICAM-3	ICAM-1	Siglec-4
E-Cadherin	ICAM-2	
ALCAM	ICAM-5	
BCAM	L-Selectin	
P-Selectin	P-Selectin	
Calectin-1		

PRECISION

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

MATERIALS PROVIDED

1. Aluminium pouches with a Microwell Plate coated with antibody to human sE-selectin (8X12)
2. 2 vials human sCD40L Standard lyophilized, 25000pg/ml upon reconstitution
3. 2 vials HRP-Conjugate solution
4. 1 bottle Standard /sample Diluent
5. 1 bottle Streptavidin-HRP Diluent
6. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
7. 1 vial Substrate Solution
8. 1 vial Stop Solution
9. 4 pieces Adhesive Films
10. package insert

STORAGE

Table 2: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 – 8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="7">May be stored for up to 1 month at 2 – 8℃.**</td></tr>
<tr><td></td></tr>
<tr><td>HRP-Conjugate solution</td></tr>
<tr><td></td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td><td rowspan="2">Standard</td><td rowspan="2">Aliquot and store for up to 1 month at -20℃.
Avoid repeated freeze-thaw cycles. Diluted standard</td></tr>
</table>

		shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 – 8℃.**

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

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000μL.
3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.

9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).
   Note: The normal human serum or plasma samples are suggested to make a 1:5 dilution.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL 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** - Reconstitute the Standard with 0.5mL of Standard /sample

Diluent. This reconstitution produces a stock solution of 50000 pg /ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 250µl of Standard/sample Diluent into the 25000 pg/ml tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 50000 pg/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℃. Diluted standard shall not be reused.

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

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

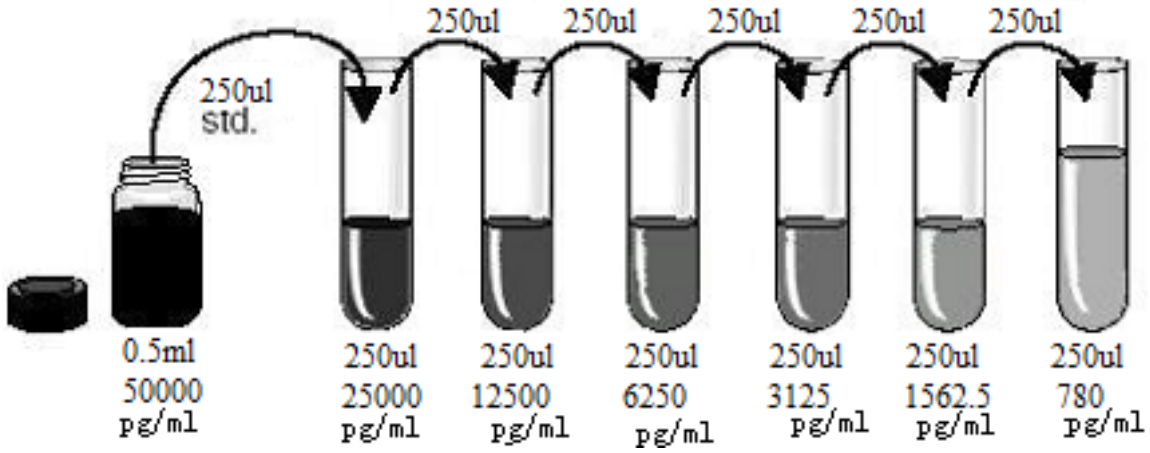

Figure 1: Preparation of sE-selectin standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.

3. Add 100µl of Standard, control, or sample, per well, then add 100µl of the working solution of HRP-Conjugate to each well. Cover with the adhesive strip provided and incubate 2 hours at RT. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µl) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100µl of Substrate Solution to each well. Incubate for 20-30 minutes at RT. Avoid placing the plate in direct light.
6. Add 100µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 650nm as the reference wave length;610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

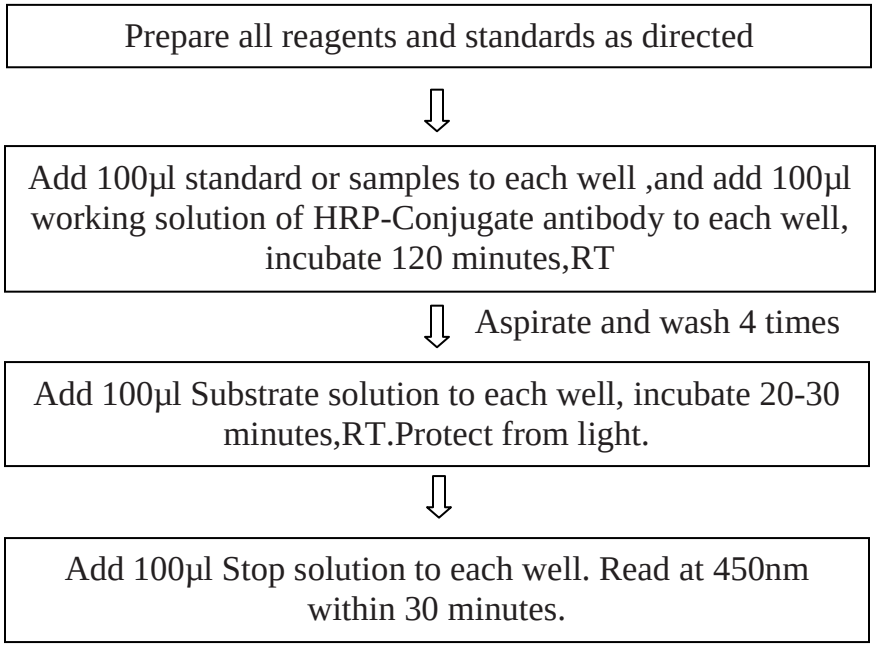

Figure 2: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create 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.
3. The data may be linearized by plotting the log of the sE-selectin concentrations versus the log of the O.D. and the best fit line can be 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 the dilution factor.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 3:Typical data using the sE-selectin ELISA (Measuring wavelength:450nm,Reference wavelength:650nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.018	0.015	0.017	——
780	0.027	0.027	0.027	0.010
1562.5	0.047	0.041	0.044	0.027
3125	0.089	0.085	0.087	0.070
6250	0.198	0.192	0.195	0.178
12500	0.405	0.408	0.407	0.390
25000	0.869	0.849	0.859	0.842
50000	1.590	1.578	1.584	1.567

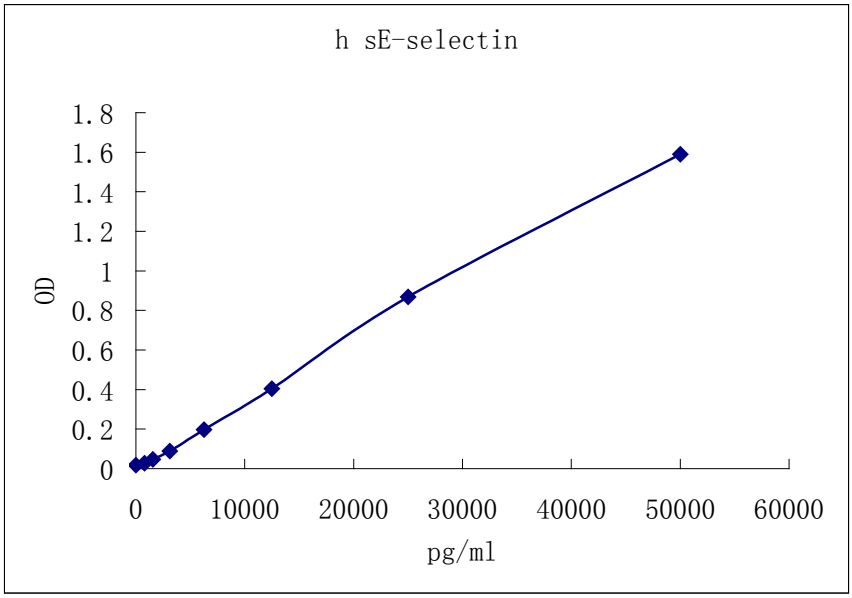

Figure 3:Representative standard curve for sE-selectin ELISA. sE-selectin was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

BACKGROUND

E-Selectin (also known as Endothelial Leukocyte Adhesion Molecule-1, ELAM-1, or CD62E) is a 115 kDa, type I transmembrane glycoprotein expressed only on endothelial cells and only after activation by inflammatory cytokines (IL-1β or TNF-α) or endotoxins (1-4). Expression is transitory, reaching a maximum within about 6 hours of stimulation and then declining with shedding of soluble E-Selectin (1-5). Cell-surface E-Selectin is a mediator of the rolling attachment of leukocytes to the endothelium, an essential step in extravasation of leukocytes at the site of inflammation (1-6) thereby playing a key role in localized inflammatory response. E-Selectin is believed to be particularly important in inflammation involving the skin (4). The extracellular part of E-Selectin includes a calcium-dependent C2-type lectin domain, an epidermal growth factor (EGF) domain, and six repeats of a complement-regulatory-protein-like sequence (1-4). E-Selectin binds sialyl Lewis X (sLex), a sialic

acid-galactose-N-acetylglucosamine-fructose tetrasaccharide, but the actual recognition is thought to be for a specific presentation of those glycosyl units in a precise three-dimensional configuration on a specific glycoprotein rather than for that particular carbohydrate (4). Soluble E-Selectin (sE-Selectin) is found in the blood of healthy individuals (7, 8), probably arising from proteolytic cleavage of the surface-expressed molecule (5, 9). Elevated levels of sE-Selectin in serum have been reported in a variety of pathological conditions (8, 10-14). Although it might be anticipated that sE-Selectin would suppress leukocyte migration by competing with surface-associated E-Selectin, it may actually activate neutrophils and act as a pro-inflammatory agent (15).

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