ELISA PRODUCT INFORMATION & MANUAL

Rat E-Selectin/CD62E ELISA Kit (Colorimetric)

NBP2-80383

Sample Insert for Reference Only

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

BACKGROUND

E-selectin, also known as endothelial leukocyte adhesion molecule-1 (ELAM-1) and CD62E, is an inducible adhesion molecule that is expressed on the surfaces of stimulated vascular endothelial cells and is sometimes involved in cancer cell metastasis. E-selectin exhibits a complex mosaic structure consisting of a large extracellular region comprised of a lectin domain, an EGF-like domain, and a short consensus repeat (SCR) domain, followed by a transmembrane region and a relatively short (32 aa) cytoplasmic tail. As a member of the LEC-CAM or selectin family, E-selectin recognises and binds to sialylated carbohydrates including members of the Lewis X and Lewis A families found on monocytes, granulocytes, and T-lymphocytes. E-selectin supports rolling and stable arrest of leukocytes on activated vascular endothelium, and furthermore, it was indicated that it can also transduce an activating stimulus via the MAPK cascade into the endothelial cell during leukocyte adhesion. E-selectin regulates adhesive interactions between certain blood cells and endothelium. The soluble form of E selectin (sE-selectin) is a marker of endothelial activation, and has a potential role in the pathogenesis of cardiovascular disease as raised levels have been found in hypertension, diabetes and hyperlipidemia, although its association in established atherosclerosis disease and its value as a prognostic factor is more controversial. soluble E-selectin is inversely associated with the muscular component of the left ventricle, thereby suggesting that the lack of such a reparative factor may be associated with cardiac remodeling in end-stage renal disease (ESRD) patients. In addition, this adhesion molecule appears to be involved in the pathogenesis of atherosclerosis.

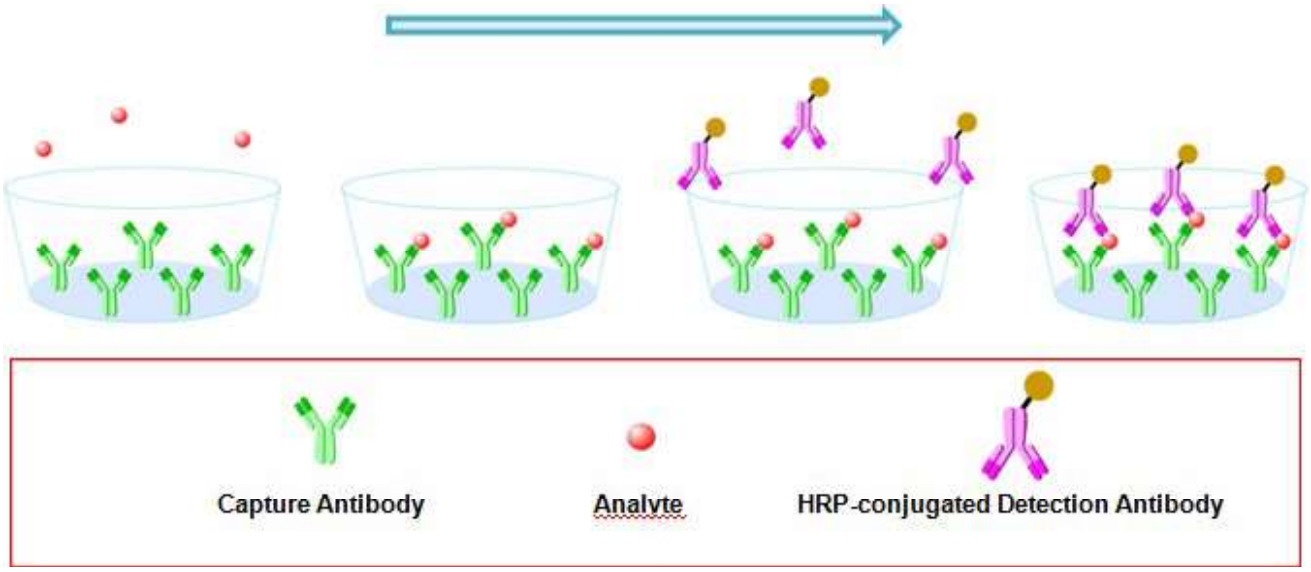
INTENDED USE

For the quantitative determination of Rat E-Selectin/CD62E concentration in serum.

The use of this kit for other sample types need be validated by the end user due to the complexity of natural targets and unpredictable interference.

PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for Rat E-Selectin/CD62E has been pre-coated onto well plate strips. Standards and samples are added to the wells and Rat E-Selectin/CD62E present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-Rat E-Selectin/CD62E antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of Rat E-Selectin/CD62E bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm (See schematics below).



MATERIALS PROVIDED

Rat E-Selectin/CD62E Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with rabbit mAb antibody against Rat E-Selectin/CD62E.

Rat E-Selectin/CD62E Detecion Antibody - 0.2 mg/mL of rabbit mAb antibody against Rat E-Selectin/CD62E conjugated to horseradish peroxidase (HRP) with preservatives.

Rat E-Selectin/CD62E Standard - Recombinant Rat E-Selectin/CD62E in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.

Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.

Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.

Color Reagent A - 13 mL of stabilized hydrogen peroxide.

Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution - 8 mL of 2 N sulfuric acid.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8℃ and the kit is stable for 6 months upon receipt.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="2">Stored for up to 1 week at 2 - 8℃</td></tr>
<tr><td>Diluted Dilution Buffer</td></tr>
<tr><td>Conjugate</td><td rowspan="3">Stored for up to 1 month at 2 - 8℃</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td><td rowspan="2">After reconstitution, store for up to 1 month at -80℃.
The reconstituted standards should be aliquoted and
avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.
Stored for up to 1 month at 2 - 8℃</td></tr>
</table>

OTHER SUPPLIES REQUIRED

·Microplate reader capable of measuring absorbance at 450 nm

·Pipettes and pipette tips

·Deionized or distilled water

·Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer

·500 mL graduated cylinder

·Tubes for standard dilution

·Well plate cover or seals

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.

2. The kit should not be used beyond the expiration date.

3. Do not mix reagents from different lots.

4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.

7. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHNICAL TIPS

8. Bring all reagents and samples to room temperature before use.

9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.

10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.

11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.

12. Read the absorbance of each well within 20 minutes after adding the stop solution.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20℃ or lower temperature. **Avoid repeated freeze -thaw cycles.**

Note:

The sample should be diluted to within the working range of the assay in 1× dilution buffer. The exact dilution must be determined based on the concentration of specific target in individual samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer - Prepare 1× wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 400 mL of Wash Buffer.

Dilution Buffer - Prepare 1× dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

Detection Antibody - Centrifuge at 10,000 x g for 20 seconds. Dilute to **work concentration** of 0.5 μg/mL in Dilution Buffer before use.

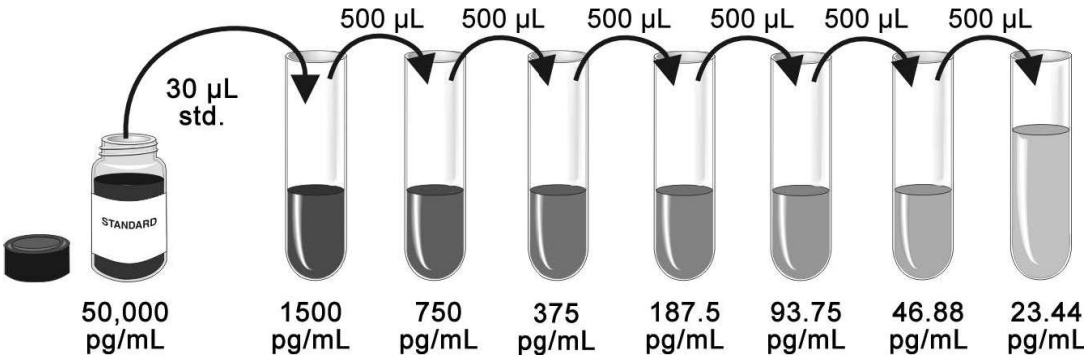
Substrate Solution **-** Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well. **Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.**

Rat E-Selectin/CD62E Standard - Reconstitute the Rat E-Selectin / CD62e / SELE Standard with 1 mL of Dilution Buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (**Do not turn the vial upside down**). Mix the standard to ensure complete reconstitution prior to making dilutions.

Prepare serially diluted standards as described in the following step:

Pipette 1000 µL of Dilution Buffer into the 1500 pg/mL tube. Pipette 500 µL of Dilution Buffer into the remaining tubes. Use the stock solution to produce a dilution series as the following figure. Mix each tube thoroughly before the next transfer. The 1500 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). **Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.**

The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous

sections.

2. Remove unused microplate strips from the plate frame, return them to the foil pouchcontaining the desiccant pack, and reseal.

3. Wash each well three times with Wash Buffer (300 µL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. **Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.**

4. Add 100 µL of each serially diluted protein standard or test sample per well including a zero standard. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate for 2 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.

6. Add 100 µL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.

8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature**. Protect from light.**

9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. **Determine the optical density of each well within 20 minutes**, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

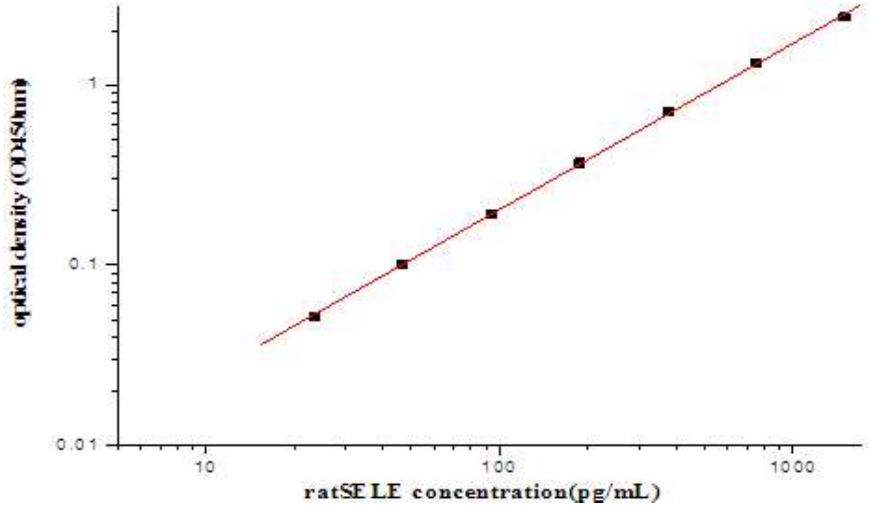
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.) .

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. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
23.44	0.052
46.88	0.101
93.75	0.193
187.5	0.369
375	0.714
750	1.318
1500	2.382



PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

	Intra -assay Precision			Inter -assay Precision		
Sample	1	2	3	1	2	3
N	20	20	20	5	5	5
Mean (pg/mL)	203	376	745	211	399	778
SD	7.34	9.68	23.14	10.62	23.96	44.70
CV (%)	3.6%	2.6%	3.1%	5.0%	6.0%	5.7%

RECOVERY

The recovery of Rat E-Selectin/CD62E spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	Average % Recovery	Range
Serum (n=3)	83%	80-88%

LINEARITY

		Serum
1:2	recovery of detected	96%
1:4	recovery of detected	101%
1:8	recovery of detected	100%
1:16	recovery of detected	103%

SENSITIVITY

The minimum detectable dose (MDD) of Rat E-Selectin/CD62E is typically less than 3.78 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant Rat E-Selectin/CD62E.

SAMPLE VALUES

The average concentration of Rat SELE in 10 normal human serum is 60.39 +/- 11.24 ng/mL ranging from 46.33 to 74.99 ng/mL.

SPECIFICITY

This assay recognizes both recombinant and natural Rat SELE. The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed.

Recombinant human			
CD62E			
Recombinant mouse			
SELE			
Recombinant rat			
IL1B	IL2	IL-10	IL12B
TNFa	IL6	IFN gama	CCL3
TIMP1	CSF2	VEGF164	SELP

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles.
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	Color Reagent should be clear and colorless prior to addition to wells	Color Reagent should be clear and colorless prior to addition to wells
	Use clean tubes and pipettes tips	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ASSAY SUMMARY