# Human sE-Selectin ELISA Kit

**Cat No. ELI-M-001-96**

Size: 96 tests

MOLEQULE-ON®

| Components | Quantity |
|---|---|
| Aluminium pouches with a Microwell Plate coated with antibody to human sE-Selectin/ CD62E (8 x 12) | 1 Plate |
| Human sE-Selectin/CD62E Standard lyophilized, 6000 pg/ml upon reconstitution | 2 Vials |
| Concentrated Biotin-Conjugate anti-human sE-Selectin/CD62E antibody | 2 Vials |
| Streptavidin-HRP solution | 2 Vials |
| Standard /sample Diluent | 4 Bottles |
| Biotin-Conjugate antibody Diluent | 1 Bottle |
| Streptavidin-HRP Diluent | 1 Bottle |
| Wash Buffer Concentrate 20x (PBS with 1% Tween-20) | 1 Bottle |
| Substrate Solution | 1 Bottle |
| Stop Solution | 1 Bottle |

## Reagent Preparations

**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. Standards** - Reconstitute the Standard with 1 ml of Standard /sample Diluent. Use 500µl of this standard in serial dilution described below (store remaining 500µl reconstituted standard at -20℃). This reconstitution produces a stock solution of 6,000pg /ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µl of Standard/sample Diluent into six tubes and marked them 3000pg/ml, 1500pg/ml, 750pg/ml, 375pg/ml, 187.5pg/ml and 93.75pg/ml respectively. Make a serial dilution by pipetting 500µl standard into first tube (3000pg/ml) and then pipette 500µl from first dilution (3000pg/ml) to second tube (1500pg/ml) and subsequently add 500µl from each tube to next tube to make serial dilution of mentioned concentration. Mix each tube thoroughly and change pipette tips between each transfer. The 6000 pg/ml standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0pg/ml). Serially diluted standard shall not be reused.

**4. Working solution of Biotin-Conjugate anti-human sE-Selectin/CD62E antibody**: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution 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.*

## Storage

All components of Human sE-selectin ELISA Kit should be store at 2-8°C except reconstituted standard that should be store at -20℃.

## Description

This kit is used for the quantitative determination of human soluble sE-Selectin (sE-Selectin) concentrations in cell culture supernatant, serum and plasma. sE-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. Soluble E-Selectin (sE-Selectin) is found in the blood of healthy individuals, probably arising from proteolytic cleavage of the surface-expressed molecule. Elevated levels of sE-Selectin in serum have been reported in a variety of pathological conditions.

## Principle

This assay based on 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.

## Sample Collection And Storage

1. **Cell Culture Supernatants**- 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.

## Procedure

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 100 µl of the working solution of HRP-Conjugate to each well. Cover with the adhesive strip provided and incubate 2 hours at room temeparture. Adequate mixing is very important for good result. Use a MOLEQULE-ON vortex 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 multichannel pipette, 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 room temperature. 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)

## Technical Note

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.

## Sensitivity, Specificity And Repeatability

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 200pg/mL.
3. **SPECIFICITY**: This assay recognizes both natural and recombinant human sE-selectin.

## References


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