HUMAN E-SELECTIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN E-SELECTIN CONCENTRATIONS IN SERUM, PLASMA AND CELL CULTURES

ELISA NAME	HUMAN ANGIOTENSINOGEN ELISA
Catalog No.	SK00523-01
Formulation	96 T
Lot No.	
Standard range	31.25-2000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma, Cell Cultures
Specificity	Human E-selectin
Calibration	Human E-Selectin rec.
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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PRODUCT INFORMATION:

DESCRIPTION

This human E-Selectin/ Endothelial leukocyte adhesion molecule-1 (ELAM-1)/ CD62E ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human E-Selectin from serum, plasma and cell cultures in a sandwich ELISA format.

This immunoassay contains recombinant human E-Selectin and Antibody raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human E-Selectin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human E-Selectin. The capture antibody can bind to the human E-Selectin in the standard and samples. After washing the plate of any unbound substances, another monoclonal antibody HRP conjugate against human E-Selectin is added to the wells. Following a wash to remove any unbound antibody enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human E-Selectin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
E-Selectin Microplate – 96 well microplate coated with an monoclonal antibody specific for human E-Selectin.	**523-01-01**	**1 plate**
E-Selectin Standard – 2 ng/vial of lyophilized recombinant human E-Selectin.	**523-01-02**	**1 vial**
Detection Antibody– 1.05 mL/vial of 10-fold concentrated solution of Anti human E-Selectin monoclonal antibody HRP conjugate	**523-01-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant Human E-Selectin.	**523-01-04**	**1 vial**
Dilution Buffer – 60 mL of buffered solution with preservative.	**DB01**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
Substrate Solution – 11 mL of substrate solution.	**SS01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 6 months. For longer storage, unopened Standard and Positive Control should be stored at -20° C or -70° C. Detection Antibody-HRP Conjugate 10-fold concentrated solution should be stored at 2 – 8° C (protect from light). Substrate Solution can be stored at 2 – 8° C for up to 6 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components can be stored at 2 – 8° C for up to 6 months. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution SHOULD BE STORED at -20° C or -70° C for up to one month.
Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

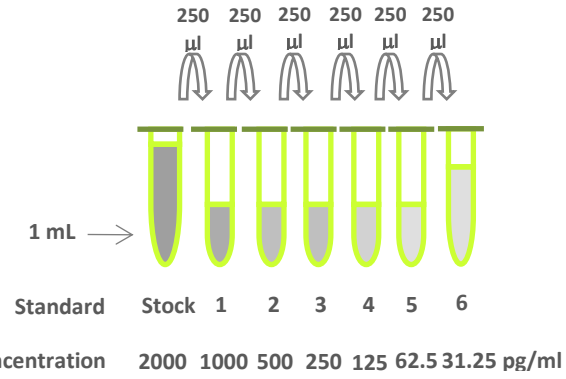
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

Human E-Selectin Standard – Reconstitute the human E-Selectin standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 2000 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1.0 mL	2000 pg/mL
# 1	200µL of stock	300µL	1000 pg/mL
# 2	250µL of 1	250µL	500 pg/mL
# 3	250µL of 2	250µL	250 pg/mL
# 4	250µL of 3	250µL	125 pg/mL
# 5	250µL of 4	250µL	62.5 pg/mL
# 6	250µL of 5	250µL	31.25 pg/mL



Positive Control - Reconstitute the Positive Control with 1 mL Dilution Buffer. **Note:** Positive Control could be used within a few days if stored at -20° C or -70° C.

Detection Antibody- Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated Detection Antibody – HRP stock solution to prepare working solution. **Note:** 1x working solution of Detection Antibody - HRP should be used within a few days **(protect from light)**. **DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Remove unneeded microplate strips from the plate frame and return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells (A2,A3).
4. Add 100 µL per well of **Standard Dilutions** in reverse order of serial dilution from #6-S (B2, B3 to H2, H3), **sample,** or **positive control** (G4, G5). Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
6. Add 100 µL per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 1 hour on microplate shaker at room temperature**. Protect from light.**
7. Repeat the aspiration and wash as in step 5.
8. Add 100 µL per well of **Substrate Solution**. Incubate for 10-15 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Read plate using a microplate reader set to 450 nm within 15 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEIN	CROSS-REACTIVITY
Human E Selectin	100%
Human EpCAM	0

TYPICAL STANDARD CURVE

This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.078)
31.25	0.046
62.5	0.091
125	0.189
250	0.341
500	0.768
1000	1.247
2000	2.240

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 1 hour on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate 10-15 min on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 15 minutes.