

RAT SOLUBLE E-SELECTIN /CD62E ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF RAT SOLUBLE E-SELECTIN
CONCENTRATIONS IN SERUM



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

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

PRODUCT INFORMATION:

ELISA NAME	RAT SOLUBLE E-SELECTIN ELISA
Catalog No.	SK00523-02
Lot No.:	
Formulation	96 T
Standard range	23-1500 pg/mL
Sensitivity	12 pg/mL
Sample Volume	100 µL
Dilution Factor	50-100 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum
Specificity	Rat E-Selectin
Calibration	Rat E-Selectin recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Rat Soluble E-Selectin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural rat E-selectin from serum in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for rat E-Selectin. The capture antibody can bind to the rat E-Selectin in the standard and samples. After washing the plate of any unbound substances, an antibody-HRP conjugate against rat E-Selectin is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of rat 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

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_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 precoated with an antibody specific for rat E-Selectin.	523-02-01	1 plate
Endoglin Standard – 1500 pg/vial of lyophilized recombinant rat E-Selectin.	523-02-02	1 vial
Detection Antibody-HRP Conjugate – 105 µL/vial of 100-fold concentrated solution of antibody conjugated to HRP against rat E-Selectin.	523-02-03	1 vial
Positive Control – one vial of lyophilized recombinant rat E-Selectin.	523-02-04	1 vial
Dilution Buffer - 60 mL of buffered solution with preservative.	DB10	1 bottle
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution.	TMB01	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, Positive Control and Detection Antibody-HRP Conjugate 100-fold concentrated solution should be stored at -20° C or -70° C. 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 microplate 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) 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 $\leq -20^{\circ}\text{C}$. 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

Rat serum samples may require a 50-100 fold dilution. A suggested 50-fold dilution is 10 μl sample + 490 μl Dilution Buffer. A suggested 100-fold dilution is 5 μl sample + 495 μl Dilution Buffer. **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 to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Rat E-Selectin Standard - Reconstitute the rat E-Selectin standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 1500 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. The **1500 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1500 pg/ml
# 1	250 μl of stock	250 μl	750 pg/ml
# 2	250 μl of 1	250 μl	375 pg/ml
# 3	250 μl of 2	250 μl	187.5 pg/ml
# 4	250 μl of 3	250 μl	93.75 pg/ml
# 5	250 μl of 4	250 μl	46.875 pg/ml
# 6	250 μl of 5	250 μl	23.44 pg/ml

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

Detection Antibody-HRP Conjugate - Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 μL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Detection Antibody-HRP conjugate 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 and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 μl per well of Dilution Buffer to Blank wells.
4. Add 100 μl per well of standard dilutions from

#6 to #S (reverse order of serial dilution), positive control or samples. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).

5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 1 hour on microplate shaker (250 rpm).
Protect from light.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 8-15 minutes on microplate shaker at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes using a microplate reader set to 450 nm.

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.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.075)
23.4	0.042
46.8	0.089
93.75	0.168
187.5	0.339
375	0.696
750	1.238
1500	2.334

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Rat E-Selectin	100%
Human E-Selectin	0

SUMMARY OF ASSAY PROCEDURE

