

# Datasheet for ABIN625209

# **L-Selectin ELISA Kit**

1 Image



## Overview

Quantity:	96 tests
Target:	L-Selectin (SELL)
Reactivity:	Chemical
Method Type:	Sandwich ELISA
Detection Range:	6-6000 pg/mL
Minimum Detection Limit:	6 pg/mL
Application:	ELISA

# **Product Details**

Purpose:	Rat L-Selectin (CD62L) ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with the following cytokines tested: rat CINC-2, CINC-3, CNTF, Fractalkine, IL-1 alpha, IL-1 beta, IL-4, IL-6, IL-10, GM-CSF, IFN-gamma, Leptin, Lix, MCP-1 MIP-3 alpha, beta- NGF, TIMP-1,TNF-alpha.
Cross-Reactivity (Details):	This ELISA kit shows no cross-reactivity with the following cytokines tested: rat CINC-2, CINC-3, CNTF, Fractalkine, IL-1alpha, IL-1beta, IL-4, IL-6, IL-10, GM-CSF, IFN-gamma, Leptin, Lix, MCP-1, MIP-3alpha, beta- NGF, TIMP-1,TNF-alpha.
Sensitivity:	< 6 pg/mL

## **Product Details**

# · Strip plates and additional reagents allow for use in multiple experiments Characteristics: · Quantitative protein detection Establishes normal range · The best products for confirmation of antibody array data · Pre-Coated 96-well Strip Microplate Components: · Wash Buffer · Stop Solution Assay Diluent(s) · Lyophilized Standard · Biotinylated Detection Antibody · Streptavidin-Conjugated HRP · TMB One-Step Substrate Material not included: · Distilled or deionized water Precision pipettes to deliver 2 μL to 1 μL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders · Tubes to prepare standard and sample dilutions · Absorbent paper

# **Target Details**

Target:	L-Selectin (SELL)
Alternative Name:	L-Selectin (SELL Products)
Background:	L-selectin (CD62 antigen-like family member L) (Leukocyte adhesion molecule 1) (LAM-1) (Leukocyte-endothelial cell adhesion molecule 1) (LECAM1) (Lymph node homing receptor) (Lymphocyte antigen 22) (Ly-22) (Lymphocyte surface MEL-14 antigen) (CD antigen CD62L)
UniProt:	P30836

· Microplate reader capable of measuring absorbance at 450nm

· Log-log graph paper or computer and software for ELISA data analysis

## **Application Details**

Application Notes:	Recommended Dilution for serum and plasma samples100 - 1,000 fold
Sample Volume:	100 μL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual.

- 2. Add 100 µL of standard or sample to each well.
- 3. Incubate 2.5 h at RT or O/N at 4 °C.
- 4. Add 100 µL of prepared biotin antibody to each well.
- 5. Incubate 1 h at RT.
- 6. Add 100  $\mu L$  of prepared Streptavidin solution to each well.
- 7. Incubate 45 min at RT.
- 8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
- 9. Incubate 30 min at RT.
- 10. Add 50 µL of Stop Solution to each well.
- 11. Read at 450 nm immediately.

#### Reagent Preparation:

- 1. Bring all reagents and samples to room temperature (18 25 °C) before use.
- 2. Sample dilution: Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of cell culture supernates. Suggested dilution for normal serum/plasma: 100-1,000 fold\*. \* Please note that levels of the target protein may vary between RayBio®Rat L-Selectin ELISA Kit Protocol 3 different specimens. Optimal dilution factors for each sample must be determined by the investigator.
- 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
- 4. Preparation of standard: Briefly spin the vial of Item C and then add  $400~\mu$ L Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates) into Item C vial to prepare a 50~ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add  $60~\mu$ L L-Selectin standard (50~ng/mL) from the vial of Item C, into a tube with  $440~\mu$ L Assay Diluent A or 1x Assay Diluent B to prepare a 6,000~pg/mL standard solution. Pipette  $400~\mu$ L Assay Diluent A or 1x Assay Diluent B into each tube. Use the 6,000~pg/mL standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Gently vortex to mix. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0~pg/mL).  $60~\mu$ L standard +  $440~\mu$ L  $200~\mu$ L
- 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. RayBio $\Re$ Rat L-Selectin ELISA Kit Protocol 4 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100  $\mu$ L of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.
- 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 500-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add

 $20~\mu\text{L}$  of HRP-Streptavidin concentrate into a tube with 10~ml 1x Assay Diluent B to prepare a 500-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well.

#### Assay Procedure:

- 1. Bring all reagents and samples to room temperature (18 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.
- 2. Add 100  $\mu$ L of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking.
- 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel pipette or autowasher. 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. RayBio®Rat L-Selectin ELISA Kit Protocol 5
- 4. Add 100  $\mu$ L of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
- 5. Discard the solution. Repeat the wash as in step
- 6. Add 100  $\mu$ L of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
- 7. Discard the solution. Repeat the wash as in step
- 8. Add 100  $\mu$ L of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
- 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

#### Calculation of Results:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Rat L-Selectin concentration (pg/mL) O D =4 50 (n m ) 0.01 0.1 1 10 100 1,000 10,000 Assay Diluent B Rat L-Selectin concentration (pg/mL) O D =4 50 (n m ) 0.01 0.1 1 10 1 10 100 1,000 10,000 RayBio®Rat L-Selectin ELISA Kit Protocol 7 Sensitivity: The minimum detectable dose of L-Selectin is typically less than 6 pg/mL. Recovery: Recovery was determined by spiking various levels of Rat L-Selectin into serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 97.06 86-107 Plasma 94.33 85-103 Cell culture media 98.42 89-107

# **Application Details**

Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 111.6 105.5 105.7 Range (%) 101-120 96-114 95-113 1:4 Average % of Expected 112.7 98.99 112.2 Range (%) 102-122 88-107 102-122 RayBio®Rat L-Selectin ELISA Kit Protocol 8 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %

Assay Precision: Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %

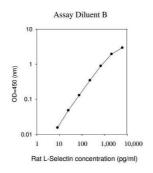
Restrictions: For Research Use only

# Handling

Expiry Date:	6 months
	recommended to store at -80°C.
	freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated
Storage:	-20 °C
Handling Advice:	Avoid repeated freeze-thaw cycles.

## **Images**

# 



### **ELISA**

Image 1.