Rat P-Selectin ELISA Kit

Catalog No.: abx255905

Size: 96T

Range: 0.313 - 20 ng/ml

Sensitivity: 0.188 ng/ml

Storage: For short term: store the whole kit at 2-8°C. For 2 to 6 months: Store only micro ELISA well plate, lyophilized standards and biotin conjugated antibody (concentrated) at -20°C and all the other components at 2-8°C

Application: For quantitative detection of P-Selectin in Rat serum, plasma or any biological fluid.

Introduction

P-selectin functions as a cell adhesion molecule (CAM) on the surfaces of activated endothelial cells, which line the inner surface of blood vessels, and activated platelets. In unactivated endothelial cells, it is stored in granules called Weibel-Palade bodies. In unactivated platelets P-selectin is stored in α-granules.

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. P-Selectin antibody is pre-coated onto 96-well plates. Biotin conjugated antibody is used as a detection antibody. The standards, test samples and biotin conjugated detection antibody are added to the wells and washed with wash buffer. HRP Streptavidin is added and unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The density of yellow is proportional to the P-Selectin amount of sample captured in plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of P-Selectin can be calculated.

Kit Components

1. One 96-well plate pre-coated with Rat P-Selectin antibody.
2. Lyophilised Rat P-Selectin standards: 2 tubes
3. Sample/Standard diluent buffer: 20ml
4. Biotin conjugated antibody (Concentrated): 120µl, Dilution 1:100
5. Antibody diluent buffer: 10ml
6. HRP streptavidin conjugate (SABC) (Concentrated): 120µl, Dilution 1:100
7. SABC diluent buffer: 10ml
8. TMB substrate: 10ml
9. Stop solution: 10ml
10. Wash buffer (25X): 30ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinder.

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

✧ **Body fluids:** Centrifuge to remove particulates, assay immediately or aliquot and store at -20°C.

✧ **Serum:** Allow the serum to clot in a serum separator tube at room temperature (30 min). Centrifuge at approximately 1000 × g for 15 minutes. Analyze the serum immediately or aliquot and store at -20°C.

✧ **Plasma** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant. Hemolysis will influence the result. Please bring samples slowly to room temperature.

General Sample guideline:

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. For high concentration, dilute 1:100, for medium concentration, dilute 1:10 and for low concentration, dilute 1:2. Very low concentrations do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

Reconstitution of the Lyophilized Rat P-Selectin standard: standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment. **(Note: Do not dilute the standard directly in the plate).**

a.) 20 ng/ml standard solution. Add **1 ml** of Sample/Standard diluent buffer into one Standard tube, keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles.

b.) 10 ng/ml → 0.32 ng/ml standard solutions: Label 6 tubes with 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.63 ng/ml and 0.32 ng/ml. Aliquot **0.3 ml** of the Sample / Standard diluent buffer into each tube. Add **0.3 ml** of the above 20 ng/ml standard solution into 1st tube and mix thoroughly. Transfer **0.3 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on.

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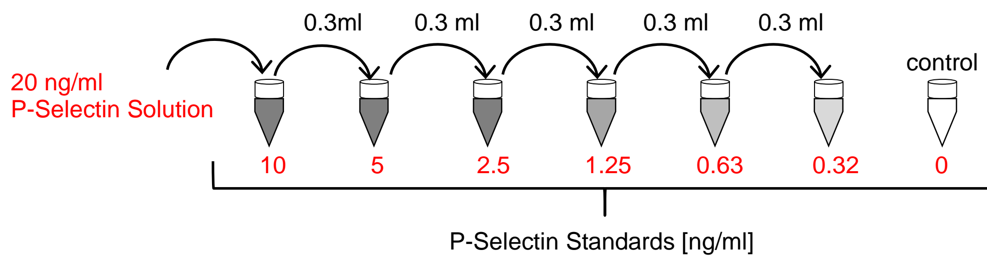

Note: The standard solutions are best used within 2 hours. The standard solution should be used within 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 1 hour before the experiment.

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the Biotin conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of Antibody diluent buffer.

5. Preparation of HRP Streptavidin Conjugate (SABC) working solution: prepare no more than 1 hour before the experiment.

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the SABC with SABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of SABC into 99 µl of SABC diluent buffer.

B. Assay procedure

Equilibrate the SABC working solution and TMB substrate for at least 30 minutes to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. Wash the plate before adding standard, samples and buffers.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37°C for 90 minutes.
6. Remove the cover and discard the contents by clapping the plate on absorbent filter papers or any other absorbent material. Do not wash the plate and do NOT let the wells dry out completely at any time.
7. Add 100 µl of Biotin conjugated antibody into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for

60 minutes.

8. Remove the cover, and wash the plate 3 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Fill each well completely with wash buffer and soak for at least 1-2 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of three times.

   Please note: For automated washing, discard the solution in all wells and wash three times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

9. Add 100 µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 minutes.

10. Remove the cover and wash the plate 5 times with Wash buffer. Allow the wash buffer to remain in the wells 1-2 min for each wash. Discard the washing buffer and blot the plate onto absorbent filter papers or other absorbent material.

11. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 25-30 minutes (incubation time is for reference only). Different shades of blue should be observed in the first 3-4 wells (with most concentrated Rat P-Selectin standard solutions). Other wells will show no obvious color.

12. Add 50 µl of Stop solution into each well and mix thoroughly. The color should change to yellow immediately.

13. Read the O.D. absorbance at 450 nm in a microplate reader within 30' of adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Rat P-Selectin concentration of the samples can be extrapolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from extrapolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
3. Avoid foaming or bubbles when mixing or reconstituting components.
4. It is recommended to assay all standards, controls and sample in duplicate or triplicate.
5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
6. To avoid cross contamination do not reuse pipette tips and tubes.
7. Do not use components from a different kit or expired ones.
8. The TMB substrate is light sensitive and should be protected from direct sunlight and UV sources. Unreacted substrate should be colorless or very light yellow in appearance. The product should be allowed to equilibrate to room temperature (25°C) prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Typical Data & Standard Curve

Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

ng/ml	0	0.32	0.63	1.25	2.5	5	10	20
OD450	0.073	0.155	0.216	0.376	0.699	1.077	1.770	2.538