



[Go to Product page](#)

Datasheet for ABIN579092

MITF ELISA Kit

1 Publication

Overview

Quantity: 96 tests

Target: MITF

Binding Specificity: Soluble

Reactivity: Rat

Method Type: Sandwich ELISA

Application: ELISA

Product Details

Purpose: This immunoassay kit allows for the specific measurement of rat soluble vascular cell adhesion molecule 1, sVCAM-1 concentrations in cell culture supernates, serum, plasma and other relevant liquid.

Sample Type: Cell Culture Supernatant, Plasma, Serum

Analytical Method: Quantitative

Detection Method: Colorimetric

Specificity: This assay recognizes recombinant and natural rat sVCAM-1.

Cross-Reactivity (Details): No significant cross-reactivity or interference was observed.

Sensitivity: The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.

Characteristics: Rattus norvegicus,Rat,Microphthalmia-associated transcription factor,Mitf

Components: Reagent (Quantity): Assay plate (1), Standard (2), Sample Diluent (1 × 20ml), Assay Diluent A

Product Details

(1x10ml), Assay Diluent B (1x10ml), Detection Reagent A (1 × 120µl), Detection Reagent B (1 × 120µl), Wash Buffer (25 x concentrate) (1 × 30ml), Substrate (1x10ml), Stop Solution (1 × 10ml)

Target Details

Target: MITF

Alternative Name: Mitf ([MITF Products](#))

Background: VCAM-1 (CD106), a member of the immunoglobulin superfamily, is a cell surface protein expressed by activated endothelial cells and certain leukocytes (such as macrophages). VCAM-1 expression is induced by IL-1beta, IL-4, TNF-alpha and IFN-gamma. VCAM-1 binds to leukocyte integrins VLA-4 and alpha4beta7. The rat and mouse SVCAM-1 proteins share approximately 76% amino acid similarity. VCAM-1 is a member of the Ig superfamily and encodes a cell surface sialoglycoprotein expressed by cytokine-activated endothelium. This type I membrane protein mediates leukocyte-endothelial cell adhesion and signal transduction, and may play a role in the development of arteriosclerosis and rheumatoid arthritis. During the inflammatory adhesion mechanism, activated integrins halt rolling leukocytes and attach them firmly to the vascular endothelium. They do this by binding to their ligands, for example VCAM-1, on endothelium. The VCAM-1: VLA-4/alpha4beta7 interaction is also thought to be involved in the extravasation of white blood cells through the blood vessel wall to sites of inflammation.

Gene ID: 3142

Pathways: [Chromatin Binding](#)

Application Details

Sample Volume: 100 µL

Plate: Pre-coated

Protocol: This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for sVCAM-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sVCAM-1 present is bound by the immobilized antibody. An enzyme-linked antibody specific for sVCAM-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of sVCAM-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 400 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (400 ng/mL). The Sample Diluent serves as the zero standard (0 ng/mL). Detection Reagent A and B - Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively.

Sample Collection: 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 and assay immediately or aliquot and store samples at -20 °C or -80 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8 °C within 30 minutes of collection. Store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. Note: Serum, plasma, and cell culture supernatant samples to be used within 7 days may be stored at 2-8 °C, otherwise samples must be stored at -20 °C (≤ 3 months) or -80 °C (≤ 6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature. It is recommended that all samples be assayed in duplicate.

Assay Procedure: Allow all reagents to reach room temperature. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Arrange and label required number of strips. Prepare all reagents, working standards and samples as directed in the previous sections.

1. Add 100 μ L of Standard, Blank, or Sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37 °C.
2. Remove the liquid of each well, don't wash.
3. Add 100 μ L of Detection Reagent A working solution to each well. Incubate for 1 hour at 37°C. Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 μ L) using a squirt bottle, multi-channel pipette, manifold dispenser 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.
5. Add 100 μ L of Detection Reagent B working solution to each well. Cover with a new adhesive

strip. Incubate for 1 hour at 37 °C.

6. Repeat the aspiration/wash as in step

5. 7. Add 90 uL of Substrate Solution to each well. Incubate within 30 minutes at 37°C. Protect from light.

8. Add 50 uL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Important Note: 1. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once.

2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

3. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.

4. Duplication of all standards and specimens, although not required, is recommended.

5. When mixing or reconstituting protein solutions, always avoid foaming.

6. 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.

7. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

Calculation of Results:

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. 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. The data may be linearized by plotting the log of the sVCAM-1 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.

Restrictions:

For Research Use only

Handling

Handling Advice:	<ol style="list-style-type: none">1. The kit should not be used beyond the expiration date on the kit label.2. Do not mix or substitute reagents with those from other lots or sources.3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.

Publications

Product cited in:	Shirpoor, Salami, Khadem-Ansari, Heshmatian, Ilkhanizadeh: "Long-term ethanol consumption initiates atherosclerosis in rat aorta through inflammatory stress and endothelial dysfunction." in: Vascular pharmacology , Vol. 57, Issue 2-4, pp. 72-7, (2012) (PubMed).
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