Datasheet for ABIN578926

MDH2 ELISA Kit

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Overview

Quantity:	96 tests
Target:	MDH2
Binding Specificity:	Soluble
Reactivity:	Rat
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of rat Soluble Intercellular Adhesion Molecule-1, sICAM-1 concentrations in cell culture supernates, serum, plasma and other biological fluids.
Sample Type:	Cell Culture Supernatant, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural rat sICAM-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,Malate dehydrogenase, mitochondrial,Mdh2,Mor1,1.1.1.37
Components:	Reagent (Quantity): Assay plate (1×20ml), Standard (2), Sample Diluent (1 × 20ml), Assay

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Product Details

	Diluent A (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 (1x10ml), Plate sealer for 96 wells (5), Instruction (1)
Material not included:	Luminometer. Pipettes and pipette tips. EP tube Deionized or distilled water.

Target Details

Target:	MDH2
Alternative Name:	Mdh2 (MDH2 Products)
Background:	Adhesion molecules mediate the interaction of cells with the extracellular matrix and with other cells. The immunoglobulin superfamily of proteins contains a large class of adhesion molecules with multiple immunoglobulin-like domains. ICAM is a member of this family. It is a 90 kDa type-I transmembrane glycoprotein with five Ig-like extracellular domains. The most important ligands for ICAM-1 are the integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), which are expressed on leukocytes. ICAM-1 thus mediates the adhesion of leukocytes to ICAM-1-expressing cells. ICAM-1 also binds fibrinogen, hyaluronan, Rhinoviruses, Plasmodium falciparum-infected erythrocytes and CD43 (sialophorin) ICAM-1 is either a transmembrane protein (mICAM-1) or soluble (sICAM-1). mICAM-1 is expressed on endothelial and epithelial cells, lymphocytes, monocytes, eosinophils, keratinocytes, dendritic cells, hematopoietic stem cells, hepatocytes and fibroblasts. Regulation of ICAM-1 expression is cell specific. Up-regulation generally is by inflammatory cytokines (TNF- alpha, IFN- gamma and IL-1) and down-regulation generally is by anti-inflammatory agents (e.g.glucocorticoids). One important, well-characterized function of ICAM-1 is immune-cell trafficking. At sites of inflammation, inflammatory cytokines induce up-regulation of ICAM-1 expression on vascular endothelial cells and activation of leukocyte integrins (LFA-1 and Mac-1). This leads to adhesion of leukocytes to the local endothelium, an essential step in migration of leukocytes to the site of inflammation. sICAM-1 has been reported in serum, cerebrospinal fluid and bronchoalveolar lavage. sICAM-1 likely arises by proteolytic cleavage of mICAM-1, synthesis from an alternatively spliced message has not been found. In general, elevated levels of serum sICAM-1 appear to be associated with inflammatory conditions and certain malignancies. It has, however, been pointed out that in inflammatory conditions, where the ligands LFA-1 and Mac-1 are likely to be activated, binding and clearance of sICAM-1 might be enhanced, so that a reciprocal relationship between sICAM-1 levels and inflammation also 2 is possible.
Gene ID:	3138

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Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to sICAM-1. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for sICAM-1 and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain sICAM-1, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of sICAM-1 in the samples is then determined by comparing the O.D. of the samples to the standard curve.
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 100 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 (100 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 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 uL 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 uL 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 uL) 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 uL of Detection Reagent B working solution to each well. Cover with a new adhesive strip.Incubate for 1 hours 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. 4

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.

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Application Details

	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 sICAM-1 concentrations versus the log of the O.D. 5 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:	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.

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