Datasheet for ABIN1112758

MMP7 ELISA Kit

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Overview

Quantity:	96 tests
Target:	MMP7
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	156-10000 pg/mL
Minimum Detection Limit:	156 pg/mL
Application:	ELISA

Product Details

Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. One 96-well plate pre-coated with anti-MMP-7 antibody 2. Standard: 0.5ml (13500pg /mL) 3. Standard diluent buffer: 1.5 ml 4. Wash buffer (30h): 20 ml.
Material not included:	1. 37 °C incubator 2. Microplate reader (wavelength: 450nm) 3. Precise pipette and disposable pipette tips 4. Automated plate washer 5. ELISA shaker 6. 1.5ml of Eppendorf tubes 7. Plate cover 8. Absorbent filter papers 9. Plastic or glass container with volume of above 1L

Target Details

Target:	MMP7
Alternative Name:	MMP-7 (MMP7 Products)
Background:	Matrix metalloproteinase-7 (MMP-7) also known as Matrilysin is an enzyme that in humans is

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	encoded by the MMP7 gene, which maps to 11q21-q22. It is a member of the MMP family, these proteins are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. This protein has 267 amino acids and is significantly shorter than stromelysin or collagenase, It was expressed in basal epithelial cells during the migration proliferation phase of corneal wound healing after excimer keratectomy. Crawford et al. (2002) hypothesized that MMP7 expression in preneoplastic pancreatic lesions applies apoptotic selective pressure to susceptible epithelial cells, resulting in selective expansion of a duct-like epithelium resistant to apoptotic stimuli.
Pathways:	Production of Molecular Mediator of Immune Response

Application Details

Comment:	This kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. The purified anti-MMP-7 antibody was pre-coated onto 96-well plates. And the HRP conjugated anti-MMP-7 antibody was used as detection antibodies. The standards test samples and HRP conjugated detection antibody were added to the wells subsequently mixed and incubated then unbound conjugates were washed away with wash buffer. TMB substrates (A & B) were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the MMP-7 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader and then the concentration of MMP-7 can be calculated.
Plate:	Pre-coated
Reagent Preparation:	1. Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes. 2. It is recommend to measure each standard and sample in duplicate. 3. Do NOT let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate. 4. Do not reuse pipette tips and tubes to avoid cross contamination. 5. Do not use the expired components and the components from different batches. 6. To avoid the marginal effect of plate incubation for temperature differences (the marginal wells always get stronger reaction), it is recommend to equilibrate the ABC working solution and TMB substrate for at least 30 min at room temperature (37°C) before adding to wells.The TMB substrate (Kit Component 8) is colorless and transparent before use, if not, please contact us for replacement.
Sample Preparation:	Preparation of sample and reagents 1. Sample Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20 °C for long term. Avoid

multiple freeze-thaw cycles.
Serum: Coagulate at room temperature for 10-20 °C min, then, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. If precipitation appeared, centrifuge again. Plasma: Collect plasma using EDTA or citrate plasma as an anticoagulant, and mix for 10-20 °C min, centrifuge at the speed of 2000-3000 r.p.m. for 20 min of collection. If precipitation appeared, centrifuge again. Urine: Collect urine using a sterile container, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. If precipitation appeared, centrifuge again. For collection of hydrothorax and cerebrospinal fluid, take reference to this operation. Cell culture supernatant: For secretory components: use a sterile container to collect. Centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. For intracellular components: Dilute cell suspension with PBS□pH7.2-7.4) to make the cell concentration reached 1 million / ml. Damage cells and release of intracellular components through repeated freeze-thaw cycles. Centrifuge at the speed of 2000-3000 r.p.m. For 20 min to collect supernatant. If precipitation appeared, centrifuge again. Tissue samples: Cut samples and weight, add certain volume of PBS (pH7.4), rapidly frozen with liquid nitrogen. After melting, store samples at 2-8°C . Add certain volume of PBS (pH7.4), homogenize thoroughly, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle. 2. NaN3 can not be used as test sample preservative, since it is the inhibitor for HRP. 3. After collecting samples, analyze immediately or aliquot and store frozen at -20 °C. Avoid repeated freeze-thaw cycles. 2. Wash buffer Dilute concentrated Wash buffer (Kit Component 4) 30-fold (1:30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water). 3. Standard Reconstitution of the Lyophilized Human MMP-7 standard (Kit Component 2): 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. 10,000pg/ml of standard solution: Add 0.5 ml of the 13,500pg/ml Standard (Kit Component 2) into 0.175ml Standard diluent buffer (Kit Component 3) and mix thoroughly. b. 5000 pg/ml to 156 pg/ml of standard solutions: Label 6 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml, 156 pg/ml, respectively. Aliquot 0.2 ml of the Standard diluent buffer (Kit Component 3) into each tube. Add 0.2 ml of the above 4000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.2 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.2 ml from 2nd tube to 3rd tube and mix thoroughly, and so on. Note: The standard solutions are best used within 2 hours. Avoid repeated freeze-thaw cycles. z Assay procedure 1. Equilibrate kit components for 15-30 min at room temperature. 2. Set test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. Do not add sample and HRP conjugated antibody to control (zero) well. 3. For test

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	sample wells, add 40myl of Sample diluent buffer first, then, add 10myl of sample. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly. 4. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37° C for 30 min. 5. Remove the sealer, and wash plate using one of the following methods: Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers. Fill each well completely with Wash Buffer (1×) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers. Repeat this procedure four more times for a total of FIVE washes. Automated Washing: Aspirate all wells, then wash plates FIVE times using Wash Buffer (1×). After the final wash, invert plate, and clap the plate on absorbent filter papers until no moisture remained. It is recommended that the washer be set for a soaking time of 10 seconds or shaking. 6. Add 50myl of HRP conjugated anti-MMP-7 antibody (Kit Component 6) to each well (except control well). 7. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37° C for 30 min. 8. Remove the sealer, and wash the plate. (See Step 5) 9. Add 50µl of TMB chromogenic reagent A (Kit Component 8) into each well, and then, add 50µl of TMB chromogenic reagent B (Kit Component 9), vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds), and incubate in dark at 37°C for 15 min. The shades of blue can be seen in the wells. 10. Add 50µl of Stop solution (Kit Component 7) into each well and mix thoroughly. The color changes into yellow immediately. 11. Read the O.D. absorbance at 450nm in a microplate reader within 15 min after 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 human MMP-7 concentration of the samples can be interpolated from the standard curve. Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution. 5000 2500 1250 625 312 156 0.2ml 0.2 ml 0.2 ml 0.2 ml 0.2 ml 0.2 ml MMP-7 Protein Standards [pg/ml] 10,000 pg/ml h. MMP-7 Solution 10,000
Restrictions:	For Research Use only

Handling

Preservative:	Sodium azide, Thimerosal (Merthiolate)