Datasheet for ABIN1379902

CCL8 ELISA Kit

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Overview

Quantity:	96 tests
Target:	CCL8
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	30-1000 pg/mL
Minimum Detection Limit:	30 pg/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows the in vitro quantitative determination of MCP-2 concentrations in cell culture supernatents, serum, plasma, tiissue lysates and urine
Sample Type:	Serum, Plasma, Urine, Cell Culture Supernatant, Tissue Lysate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1.One 96-well plate pre-coated with anti-human MCP-2 antibody 2. Standard: 0.5mL (1350pg /mL) 3. Standard diluent buffer: 1.5 mL 4. Wash buffer (30×): 20 mL. Dilution: 1:30 5. Sample diluent buffer: 6 mL

Product Details

	6. HRP conjugated anti-human MCP-2 antibody (RTU): 6mL 7. Stop solution: 6 mL 8. TMB substrate A: 6mL 9. TMB substrate B: 6mL 10. Plate sealer: 2 11. Hermetic bag: 1
Material not included:	1.37*℃ 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.Absorbent filter papers 8.Plastic or glass container with volume of above 1L

Target Details

Target:	CCL8
Alternative Name:	MCP-2 (CCL8 Products)
Background:	Monocyte chemotactic protein 2 (MCP-2), also known as Chemokine (C-C motif) ligand 8 (CCL8), is a CC chemokine that utilizes multiple cellular receptors to attract and activate human leukocytes. CCL8 is a small cytokine belonging to the CC chemokine family. It is a potent inhibitor of HIV-1 by virtue of its high-affinity binding to the receptor CCR5, one of the major co-receptors for HIV-1. It elicits its effects by binding to several different cell surface receptors called chemokine receptors. These receptors include CCR1, CCR2B and CCR5

Application Details

Plate:	Pre-coated
Protocol:	This kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. The purified anti-MCP-2 antibody was pre-coated onto 96-well plates. And the HRP conjugated anti-MCP-2 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 MCP-2 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of MCP-2 can be calculated.

Sample Preparation:

1.Sample Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20*℃ for long term. Avoid multiple freeze-thaw cycles.*
Serum: Coagulate at room temperature for 10-20 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 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 *℃ . 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.NaN 3 cannot 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 Dilution of the Human MCP-2 standard (Kit Component 2): standard solution should be prepared no more than 2 hours prior to the experiment. (
Note: Do not dilute the standard directly in the plate) a. 1000 pg/mL of standard solution: Add

	0.5 mL of the 1350pg/mL Standard (Kit Component 2) into 0.175mL Standard diluent buffer (Kit Component 3) and mix thoroughly. b. 1000 pg/mL -> 31.3 pg/mL of standard solutions: Label 5 Eppendorf tubes with 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 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 1000 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.
Assay Procedure:	1.Equilibrate kit components for 15-30 min at room temperature. 2.Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. Add 50µl of diluted standards (1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL) into the standard wells. Add 100µl of Standard diluent buffer (Kit Component 3) into the control (zero) well. Do not add sample and HRP conjugated antibody into the control (zero) well. 3.For test sample wells, add 40µl of Sample diluent buffer (Kit component 5) first, then, add 10µl 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 50µl of HRP conjugated anti-MCP-2 antibody (Kit Component 6) into 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

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	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)
Restrictions:	For Research Use only

Handling

Preservative:	Sodium azide, Thimerosal (Merthiolate)
Handling Advice:	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. Store the TMB substrate A & B (Kit Component 8 & 9) in dark. 7. Prolong the incubation time if the hypochromasia obtained. Heat the water in the water bath during diluting if the crystalloid appeared in Wash buffer (Kit Component 4). 8. Do not remove microplate from the storage bag until needed, and the unused strips should be stored at 2-8*°C in their pouch or the provided Hermetic bag (Kit Component 11).*
Storage:	4 °C
Expiry Date:	6 months

Publications

Product cited in:	Blaszczyk, Coillie, Proost, Damme, Opdenakker, Bujacz, Wang, Ji: "Complete crystal structure of monocyte chemotactic protein-2, a CC chemokine that interacts with multiple receptors." in: **Biochemistry**, Vol. 39, Issue 46, pp. 14075-81, (2000) (PubMed).