

Datasheet for ABIN1112666
Lipocalin 2 ELISA Kit



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Overview

Quantity:	96 tests
Target:	Lipocalin 2 (LCN2)
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	156-10000 pg/mL
Minimum Detection Limit:	156 pg/mL
Application:	ELISA

Product Details

Purpose:	For quantitative detection of NGAL in mouse serum, plasma, urine, cell culture supernatant or tissue samples.
Sample Type:	Serum, Plasma, Urine, Tissue Samples, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. One 96-well plate pre-coated with anti-mouse NGAL antibody 2. Standard: 0.5ml (13500pg /mL) 3. Standard diluent buffer: 1.5 ml 4. Wash buffer (30x): 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:	Lipocalin 2 (LCN2)
Alternative Name:	NGAL / Lipocalin (LCN2 Products)
Background:	Lipocalin-2 (LCN2), also known as oncogene 24p3 or neutrophil gelatinase-associated lipocalin (NGAL), is a protein associated with neutrophil gelatinase. The 25-kD LCN2 protein is believed to bind small lipophilic substances such as bacteria-derived lipopolysaccharide (LPS) and formylpeptides and may function as a modulator of inflammation. Upon encountering invading bacteria the toll-like receptors on immune cells stimulate the synthesis and secretion of lipocalin-2. Secreted lipocalin-2 then limits bacterial growth by sequestering iron-containing siderophores. Lipocalin-2 also functions as a growth factor.
Pathways:	Cellular Response to Molecule of Bacterial Origin , Transition Metal Ion Homeostasis

Application Details

Comment:	This kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. The purified anti-NGAL antibody was pre-coated onto 96-well plates. And the HRP conjugated anti-NGAL 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 NGAL amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader and then the concentration of NGAL 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 Mouse NGAL 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 → 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. Chongqing Biospes Co., Ltd Product Manual

Restrictions: For Research Use only

Handling

Preservative: Sodium azide, Thimerosal (Merthiolate)