Datasheet for ABIN5664985

CD14 ELISA Kit

1 Image | 1 Publication

Go to Product page

Overview

Quantity:	96 tests
Target:	CD14
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	3 ng/mL - 50 ng/mL
Minimum Detection Limit:	3 ng/mL
Application:	ELISA

Product Details

Purpose:	The mouse CD14 kit has been developed for the quantitative measurement of natural and recombinant mouse CD14 in serum, plasma and culture medium.
Sample Type:	Plasma, Serum, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	No reaction with human, rabbit, horse, pork, bovine or rat CD14 antibodies
Sensitivity:	Normal CD14 range in healthy mice: (0.3 - 6ug/ml) n= 10, Interassay variation coefficient: 9.8% till 17.8 depending of concentration, Intraassay variation coefficient: 6.9%, n=10 serum samples, Effective range: 5 -50 ng/ml
Characteristics:	A mixture of monoclonal antibodies specific for mouse sCD14 is coated at modules. In the first step the precoated modules will be incubated with the antigen (standard or sample) together with a POD-labelled antibody specific for mouse sCD14. During this incubation, mouse CD14 is

Product Details

	captured by solid bound antibody. Unbound material present in the sample is removed by washing. Revelation step includes TMB as chromogen. The enzyme reaction is stopped by the addition of sulphuric acid (O.25M) and the absorption at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The mouseCD14 concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve. The dilution step of sample with second antibody is incorporated in standard curve.
Components:	1x Precoated ELISA modules, detecting antibody (POD-labelled monoclonal antibody), Mouse CD14-standard, Reference serum, PBS, Dilution Buffer ,Tween 20, Stopping solution, Substrate solution
Material not included:	Orbital shaker, Micro plate reader for measurement absorbance at 450 /620 nm, Precision pipettes with disposable tips, 10-1000 ul adjustable multiwell pipettes

Target Details

Target:	CD14
Alternative Name:	CD14 (CD14 Products)
Background:	Background: The CD14 glycoprotein, gp 55, is present on most monocytic and macrophages like cell types: monocytes, macrophages, weekly at surface of neutrophiles like Kupffer cells, pleural phagocytic cells and dendritic reticular cells. CD14 is also observed on granulocytes and activated or transformed B-cells. Furthermore CD14 is present in a soluble form in human serum, urine and other body fluids. The CD14 Molecule has been reported to be a receptor for endotoxin. CD14 is anchored to cells by linkage to glycosylphosphatidylinositol (GPI) and functions as a high affinity receptor for LPS-LBP (lipopolysaccharide binding protein)-complexes.
Molecular Weight:	~50kDa
Gene ID:	12475
NCBI Accession:	NP_033971
UniProt:	P10810
Pathways:	TLR Signaling, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Toll-Like Receptors Cascades

Application Details

Application Notes:	**Preparation of reagents:** Use all reagents for assay at room temperature. (A) Wash Buffer: Dissolve 1 tablet PBS (vial 5) in 200 ml distilled water-add 100 ul Tween 20 (vial 7) , store at room temperature. Prepared wash buffer is stable for 4 weeks at refrigerator. (B) PBS: (Phosphate balanced salt solution) Dilute 1 tablet of vial 5 in 200 ml distilled water. Store and use at room temperature. (C) Sample dilution buffer: Dissolve content of vial 6 with 50 ml PBS (Buffer C) and add 50ul Tween 20 from vial 7. Use buffer at room temperature. This buffer is 1-2 weeks stable at 4oC. (D) Detecting ab dilution buffer: Add whole content of the vial10 to 10ml PBS (Buffer B). Prepare just before use. Store remaining buffer after reconstitution at -20oC. (E) Detection antibody: Add 500 ul detecting ab dilution buffer (D) to vial 2, mix carefully and than dissolve 250ul of this vial 2 in 8 ml dilution buffer for detecting ab (D). Prepare just before use. (F) Reference mouse serum lyophilized: Add 10ul distilled water to vial 4 for solubility and secondly dilute the whole content of vial 4 with 1490 ul dilution buffer for samples in a new vial (C). Pipette 50ul/well. This represents a dilution of 1:150. The mCD14 content of this reference serum is 2.7
Sample Volume:	100 µL
Assay Time:	2 h
Plate:	Pre-coated
Protocol:	The sCD14 Kit is a solid phase sandwich Enzyme-Linked-Immunosorbent-Assay (ELISA). A mixture of monoclonal antibodies specific for mouse sCD14 is coated at modules. In the first step the precoated modules will be incubated with the antigen (standard or sample) together with a POD-labelled antibody specific for mouse sCD14. During this incubation, mouse CD14 is captured by solid bound antibody. Unbound material present in the sample is removed by washing. Revelation step includes TMB as chromogen. The enzyme reaction is stopped by the addition of sulphuric acid (O.25M) and the absorption at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The mouseCD14 concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve. The dilution step of sample with second antibody is incorporated in standard curve.
Reagent Preparation:	A Wash Buffer: Dissolve 1 tablet PBS (vial 5) in 200 mL distilled water-add 100 µL Tween 20 (vial 7) , store at room temperature. Prepared wash buffer is stable for 4 weeks at refrigerator. B PBS: (Phosphate balanced salt solution) Dilute 1 tablet of vial 5 in 200 mL distilled water. Store and use at room temperature. C Sample dilution buffer: Dissolve content of vial 6 with 50 mL PBS (Buffer C) and add 50 µL Tween 20 from vial 7. Use buffer at room temperature. This

	buffer is 1-2 weeks stable at 4°C. D Detecting ab dilution buffer: Add whole content of the vial10 to 13 mL PBS (Buffer B). Prepare just before use. Store remaining buffer after reconstitution at -20°C E Detecting antibody: Firstly add 500 µL dilution buffer for detecting antibody (D) to vial 2 for solubility (=0.23 µg/mL IgG) , mix carefully and secondly add the 250 µL of this vial 2 in a new vial containing 12 mL of D. Prepare just before use. I F Reference mouse serum lyophilized: Add 10 µL distilled water to vial 4 for solubility and secondly dilute the whole content of vial 4 with 2990 µL dilution buffer for samples (C) in a new vial. Pipette 50 µL/well. This represents a dilution of 1:300. The mCD14 content of this reference serum is 4.3 ± 2.2 µg/mL. G Mouse CD14-standard lyophilized: Firstly pipette 30 µL distilled water to the vial 3 for reconstitution and secondly pipette the whole reconstituted content of vial 3 in a new vial with 770 µL sample dilution buffer (C) and mix carefully. This is vial a. For standard curve prepare vial b-f. Prepare just before use.
Sample Collection:	Serum, plasma and other human LBP containing solutions are suitable for use in the test.Samples containing a visible precipitate must be clarified prior to use in the assay. Lipemic and haemolysed probes are not possible. Samples should be frozen at -20°C for a long-term storage.
Sample Preparation:	The mouseCD14 concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve. The dilution step of sample with second antibody is incorporated in standard curve.
Assay Procedure:	ASSAY PROCEDURE FOR ""ONE STEP"" ASSAY Let all reagents reach room temperature and mix thoroughly 1. Samples and detecting antibody Add 50 µL of standards (G) vial b-f (50, 25, 12.5, 6.25, 3.12 ng/mL), reference (F) or diluted samples in duplicate into the corresponding wells as well as 50 µL detecting antibody (E). Incubate for 1.5 hours at room temperature with shaking. 2. 3 x washing with 250 µL Wash Buffer/well (A). Remove the Wash Buffer carefully after each wash. 3. Substrate Add 100 µL Substrate (vial 9) to each well. Incubate 14 ± 1 min at room temperature without shaking in the dark up to strong colour change to blue is visible. 4. Stopping Add 100 µL stopping solution (vial 8) to each well. Tape plate gently to mix, now colour is yellow 5. Read absorbance of wells at 450 nm (reference wave length 620).
Calculation of Results:	Remediate the optical density (OD) with blank, calculate the mean of corrected OD of standard duplicates, reference serum and the samples. Design a standard curve by plotting the OD means of standards (a-f) (y-axis) and the LBP concentration (x-axis). Calculate the LBP concentration from the mean OD of samples from the standard curve and multiply with dilution factor.
Assay Precision:	interassay vc 10%, intra assay vc 6%

Application Details

Restrictions:	For Research Use only

Handling

Preservative:	Without preservative
Precaution of Use:	protect your eyes
Storage:	4 °C
Storage Comment:	Short time store at 2-8°C, Long time storage of lyophilized reference serum and standard at -20°C or -80°C, detecting monoclonal can be stored at 2-8°C

Publications

Product cited in:	Alaish, Smith, Timmons, Greenspon, Eyvazzadeh, Murphy, Shea-Donahue, Cirimotich, Mongodin, Zhao, Fasano, Nataro, Cross: "Gut microbiota, tight junction protein expression, intestinal resistance, bacterial translocation and mortality following cholestasis depend on the genetic background of the host." in: **Gut microbes**, Vol. 4, Issue 4, (2013) (PubMed).

Images

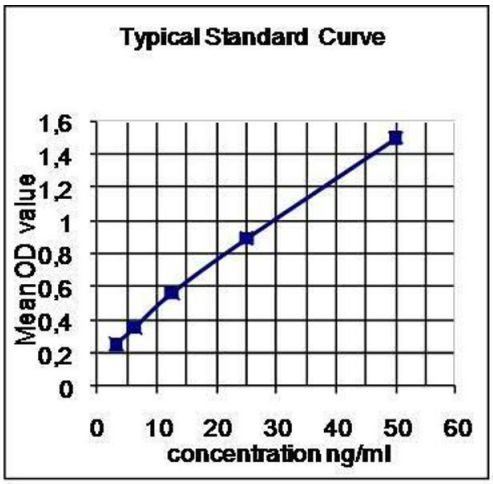

Image 1.

