Datasheet for ABIN5664984

CD14 ELISA Kit

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Overview

Quantity:	96 tests
Target:	CD14
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1.5 ng/mL - 50 ng/mL
Minimum Detection Limit:	1.5 ng/mL
Application:	ELISA

Product Details

Purpose:	The Human CD14 kit has been developed for the quantitative measurement of natural and recombinant soluble human CD14 (sCD14) in serum, plasma and culture medium.
Sample Type:	Plasma, Serum, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	No cross reaction with mouse, rabbit, horse, pork, bovine or rat CD14 antibodies
Sensitivity:	Normal CD14 range in healthy blood donors: (1.79-3.68 ug/ml) n= 10. Interassay variation coefficient: 9.8 till 11.8 depending of concentration. Intraassay variation coefficient: 4.9%, n=10 serum samples. Effective range: 5 -50 ng/ml
Characteristics:	A mixture of two monoclonal antibodies specific for sCD14 is coated to modules. In the first step the precoated modules will be incubated with the antigen (standard or sample). During this incubation, human CD14 is captured by solid bound antibody. Unbound material present in the

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	sample will be removed by washing. Then a POD-labelled monoclonal antibody specific for sCD14 is incubated. Revelation step includes TMB as chromogen. The enzyme reaction is stopped by the addition of 0.25 Mol sulphuric acid and the absorption is measured at 450 nm with a spectrophotometer. A standard curve will be provided by plotting the absorptions versus the corresponding concentrations of the known standards. The humanCD14 concentration of samples with unknown concentrations, running parallel with the standards, can be determined from the standard curve.Serum, plasma and other CD14 containing solutions are suitable for use in the test. With coagulation inhibitor citrate the CD14 content is lower then with EDTA or heparin. 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. Depending on the concentration of sCD14 in the samples, these have to be diluted with dilution buffer. For normal serum samples a dilution of 1:200 is recommended.
Components:	1x Precoated ELISA modules, detecting antibody (POD-labelled monoclonal antibody), Human 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:	929
NCBI Accession:	NP_000582

Target Details

UniProt:	P08571
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 (recommendations for 1 plate):** (A) Wash Buffer: PBS/ Tween 0.05%: Dissolve 1 Tablet Phosphate buffered saline (PBS, vial 5) in 200 ml distilled water and add 100ul Tween 20 (,vial 7). (Prepared wash buffer is stable for 4 weeks at refrigerator). (B) PBS: Dilute 1 Tablet of vial 5 in 200 ml distilled water. (C) Dilution buffer: Dissolve content of vial 6 with 50 ml PBS (Buffer B) and add 50ul Tween 20 from vial 7. This buffer is stable for 1-2 weeks at 4oC. Attention! Use buffer for assay at room temperature. (D) Reference serum: For reconstitutions of lyophilized reference serum add 10 ul distilled water and than dilute with 1990ul Dilution buffer (C) For testing use 100 ul /well. Reference serum contained 3.2
Sample Volume:	100 µL
Assay Time:	2.5 h
Plate:	Pre-coated
Protocol:	The sCD14 kit is a solid phase sandwich Enzyme-Linked-Immuno- Sorbent-Assay (ELISA). A mixture of two monoclonal antibodies specific for sCD14 is coated to modules. In the first step the precoated modules will be incubated with the antigen (standard or sample). During this incubation, human CD14 is captured by solid bound antibody. Unbound material present in the sample will be removed by washing. Then a POD-labelled monoclonal antibody specific for sCD14 is incubated. Revelation step includes TMB as chromogen. The enzyme reaction is stopped by the addition of 0.25 Mol sulphuric acid and the absorption is measured at 450 nm with a spectrophotometer. A standard curve will be provided by plotting the absorptions versus the corresponding concentrations of the known standards. The humanCD14 concentration of samples with unknown concentrations, running parallel with the standards, can be determined from the standard curve.
Reagent Preparation:	Reagents A Wash Buffer: PBS/ Tween 0.05 % : Dissolve 1 tablet phosphate buffered saline (PBS, vial 5) in 200 mL distilled water and add 100 µL Tween 20 (vial 7). Prepared wash buffer is stable for 4 weeks at refrigerator. B PBS: Dilute 1 tablet of vial 5 in 200 mL distilled water C Dilution buffer: Dissolve content of vial 6 with 50 mL PBS (Buffer B) and add 50 µL Tween 20 from vial 7. This buffer is stable for 1-2 weeks at 4°C. Attention! Use buffer for assay at room temperature. D Reference serum: For reconstitutions of lyophilized reference serum add 10 µL

	distilled water and than dilute with 1990 µL Dilution buffer (C). This represents dilution 1:200. For test use 100 µL /well. Reference serum contained 3.2 ± 0.6 µg/mL solubleCD14. E CD14-standard: 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 (vial 0) together with 970 µL Dilution buffer (C) and mix carefully. Now use 50 µL of vial 0 and add 450 µL Dilution buffer (C).This represents = vial a with CD14 concentration of 50 ng/mL. For standard curve prepare and use vial a -e Prepare just before use. Store the standard at - 20°C.
Sample Collection:	Serum, plasma and other CD14 containing solutions are suitable for use in the test. With coagulation inhibitor citrate the CD14 content is lower then with EDTA or heparin. 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. Depending on the concentration of sCD14 in the samples, these have to be diluted with dilution buffer. For normal serum samples a dilution of 1:200 is recommended. Serum, plasma and other CD14 containing solutions are suitable for use in the test. With coagulation inhibitor citrate the CD14 content is lower then with EDTA or heparin. 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. Depending on the concentration of sCD14 in the samples, these have to be diluted with dilution buffer. For normal serum samples a dilution of 1:200 is recommended. Serum, plasma and other CD14 containing solutions are suitable for use in the test. With coagulation inhibitor citrate the CD14 content is lower then with EDTA or heparin. 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. Depending on the concentration of sCD14 in the samples, these have to be diluted with dilution buffer. For normal serum samples a dilution of 1:200 is recommended. Serum, plasma and other CD14 containing solutions are suitable for use in the test. With coagulation inhibitor citrate the CD14 content is lower then with EDTA or heparin. 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. Depending on the concentration of sCD14 in the samples, these have to be diluted with dilution buffer. For normal serum samples a dilution of 1:200 is recommended.
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Assay Procedure:	ASSAY PROCEDURE Let all reagents reach room temperature and mix thoroughly 1. Samples Add 100 µL of standards (50, 25, 12.5, 6.25, 3.12 ng/mL= vials a-e) or diluted samples in duplicate into the corresponding wells and incubate for one hour at room temperature and shaking. 2. 3 x washing with Wash Buffer (A). 3. Detecting antibody Add 100 µL detecting antibody (vial 2) to each well and incubate at room temperature for 1 hour at shaker. 4. 3 x washing with Wash Buffer (A). 5. Substrate Add 100 µL Substrate solution (vial 9) to each well. Incubate 13 ± 1 min at room temperature without shaking in the dark. 6. Stopping Add 100 µL stopping solution (vial 8) to each well. Tape plate gently to mix 7. 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 5%
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

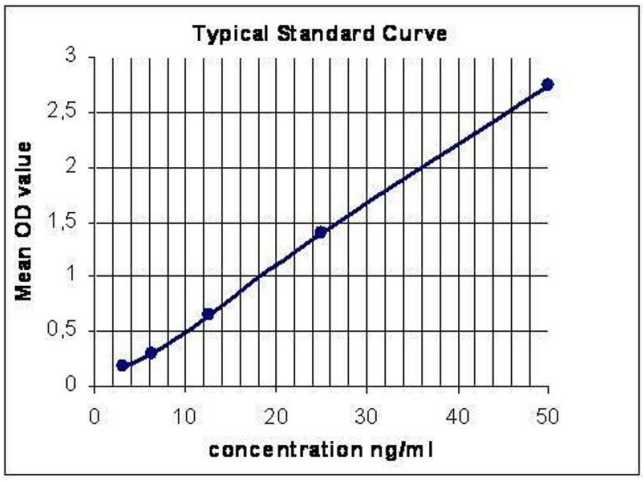

Image 1.

