CD14 (Human) ELISA Kit

Catalog Number KA0446

96 assays

Version: 04

Intended for research use only

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Introduction

Principle of the Assay

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. 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 human CD14 concentration of samples with unknown concentrations, running parallel with the standards, can be determined from the standard curve.

General Information

Materials Supplied

List of component

1	Precoated ELISA modules	1 plate
Vial 2	Detecting antibody (POD-labelled monoclonal antibody to human CD14) ”Ready for use”	1 vial
Vial 3	Human-CD14-standard (recombinant human CD14, lyophilized)	1 vial
Vial 4	Reference serum (3.2 ± 0.6 µg/mL, lyophilized)	1 vial
Vial 5	PBS (Phosphate buffer salt solution)	2 tab.
Vial 6	Dilution Buffer	1 vial
Vial 7	Tween 20	1 vial
Vial 8	Stopping solution “Ready for use”	1 vial
Vial 9	Substrate solution “Ready for use”	1 vial

Storage Instruction

Short time store at 2-8°C, Long time storage of vials 3-4 at -20°C or -80°C.

The test kit is stable for some days at room temperature as well as 3 days at 37°C.

Materials Required but Not Supplied

- ✓ Orbital shaker
- ✓ Micro plate reader for measurement absorbance at 450 nm/620 nm
- ✓ Precision pipettes with disposable tips
- ✓ 10-1000 µL adjustable multiwell pipettes

Assay Protocol

Reagent Preparation

- Wash Buffer:
  PBS/Tween 0.05%: Dissolve 1 Tablet Phosphate buffered saline (PBS, provided) in 200 mL distilled water and add 100 µL Tween 20 (provided). Prepared wash buffer is stable for 4 weeks at refrigerator.
- PBS: Dilute 1 Tablet of PBS (provided) in 200 mL distilled water
- Dilution buffer: Dissolve content of Dilution buffer (provided) with 50 mL PBS and add 50 µL Tween 20. This buffer is 1-2 weeks stable at 4°C. Attention! Use buffer for assay at room temperature.
- Reference serum: For reconstitution of lyophilized reference serum add 10 µL distilled water and than dilute with 1990 µL Dilution buffer. This represents dilution 1:200. For test use 100 µL /well. Reference serum contained 3.2 ± 0.6 µg/mL soluble CD14.
- CD14-standard: Firstly pipette 30 µL distilled water to the Human-CD14-standard for reconstitution and secondly pipette the whole reconstituted content of Human-CD14-standard in a new vial (vial 0) together with 970 µL Dilution buffer and mix carefully. Now use 50 µL of vial 0 and add 450 µL Dilution buffer. This represents = vial a with CD14 concentration of 50 ng/mL.
  For standard curve prepare and use vial a – e

. No	CD14-Standard dilution µL	Dilution buffer	Concentration ng/mL
vial a			50
vial b	250 µL of vial a	250 µL	25
vial c	250 µL of vial b	250 µL	12.5
vial d	250 µL of vial c	250 µL	6.25
vial e	250 µL of vial d	250 µL	3.125

Prepare just before use. Store the standard at -20°C.
Mix vials 2, 8 and 9 (“Ready for use”) carefully before use!!!

Sample Preparation

- ✓ 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.

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= vial 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.
3. Detecting antibody
   Add 100 µL detecting antibody to each well and incubate at room temperature for 1 hour at shaker.
4. 3 x washing with Wash Buffer.
5. Substrate
   Add 100 µL substrate solution to each well. Incubate 13 ± 1 min at room temperature without shaking in dark.
6. Stopping
   Add 100 µL stopping solution to each well. Tape plate gently to mix.
7. Read absorbance of wells at 450 nm (reference wave length 620 nm)

Data Analysis

Calculation of Results

Calculate the mean of optical density (OD) of standard duplicates, reference serum and the samples. Design a standard curve by plotting the OD means of the standards (a-e) (y-axis) and the CD14 concentration (x-axis). Calculate the CD14-concentration from the mean OD of the samples from the standard curve and multiply with dilution factor.

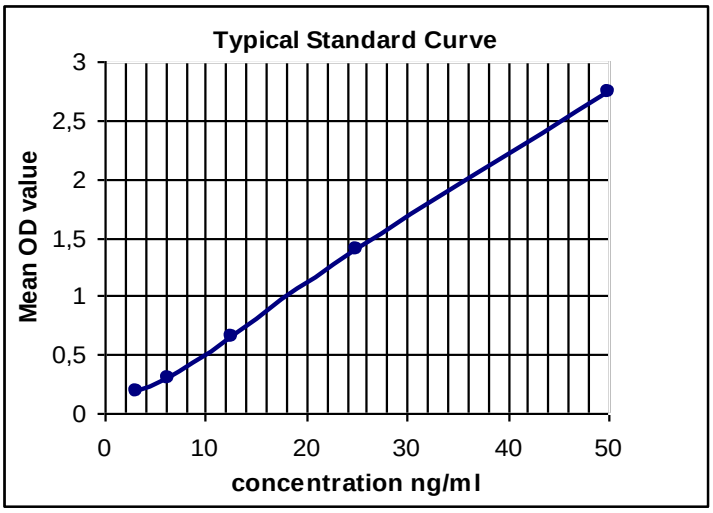

Figure 1: Typical Standard Curve for CD14 (Human) ELISA Kit.

Performance Characteristics

- Normal CD14 range in healthy blood donors: (1.79-3.68 µg/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
- Cross reaction: unknown

Resources

References

1. R.Bregadze: Untersuchungen zur Assoziation genetischer Polymophismen im Gen des Endotoxinreceptors CD14 mit der transkriptionellen Aktivitat Inaugural-Diss. Univ. Gottingen 2010

Plate Layout

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