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Human sCD14 ELISA Kit

Catalog No: CKH114 **Size:** 1 x 96 tests **Lot No:**

Introduction

The human sCD14 kit has been developed for the quantitative measurement of natural and recombinant human CD14 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 pre-coated to the modules of the plate. In the first step the pre-coated 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. Next, a POD-labelled monoclonal antibody specific for sCD14 is incubated. The detection 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 is obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The human CD14 concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined by the standard curve.

Reagents and materials supplied with the kit:

1	Precoated ELISA modules	1 plate
Vial 2	Detection antibody (POD-labeled monoclonal antibody to human CD14) "Ready for Use"	1 vial
Vial 3	CD 14-standard (recombinant human CD14, lyophilized)	1 vial
Vial 4	Reference serum (3.2 ± 0.6, lyophilized)	1 vial
Vial 5	PBS	2 Tablets
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

Note: Store at 2-8°C short term. Long term storage of vials 3-4 at -20ºC or -80ºC.
Kit is stable for several days at room temperature and for 3 days at 37°C.

Material required but not provided:

- Orbital shaker
- Micro plate reader for measurement of absorbance at 450 nm and 620 nm
- Precision pipettes with disposable tips
- 10-1000 µl adjustable multi-well pipettes

Cell Sciences, Inc.
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 578 572-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

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Preparation of reagents

A Wash Buffer

- PBS/Tween 0.05%
- Dissolve one tablet Phosphate Buffered Saline (PBS, vial 5) in 200 ml distilled water
- Add 100 µl of 0.05 % Tween 20 (vial 7).

Prepared wash buffer is stable for 4 weeks refrigerated.

B PBS

- Dilute one tablet of vial 5 in 200 ml distilled water

C Dilution buffer

- Dissolve contents of vial 6 with 50 ml PBS (Buffer B) and add 50 µl Tween 20 from vial 7.
- This buffer is stable at 2-4°C for one to two weeks.
- **Note:** Use buffer for assay at **room temperature**.

D Reference Serum

- For reconstitution of lyophilized reference serum add 10 µl distilled water, then add 1990 µl Dilution buffer (C)
- For testing, use 100 µl/well. Reference Serum contained 3.2 ± 0.6 µg/ml.

E CD14-standard

- First, pipette 30 µl distilled water to vial 3 for reconstitution. Second, pipette the whole reconstituted contents of vial 3 into a new vial (vial 0) with 970 µl Dilution Buffer (C) and mix carefully.
- 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.

No	CD14-Standard Dilution µl	Dilution buffer (D)	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**.
Note: Mix Vials 2, 8 and 9 ("Ready for Use" vials) carefully before using.

Preparation of Samples

The human sCD14 kit has been developed for the quantitative measurement of natural and recombinant human serum, plasma and other sCD14 containing solutions are suitable for use in the test. With coagulation inhibitor citrate the sCD14 content is lower than with EDTA or heparin. Samples containing a visible precipitate must be clarified prior to use in the assay. Lipemic and hemolyzed probes are not possible.

Samples should be frozen at -20°C for 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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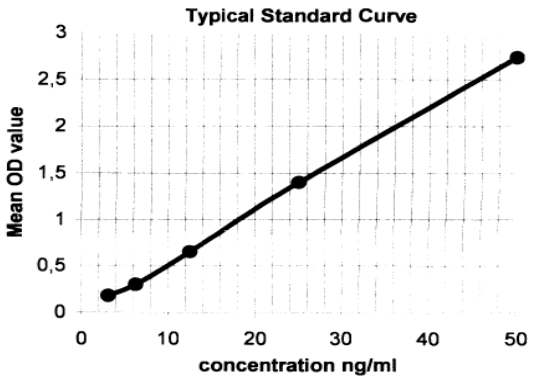
Assay Characteristics:

Normal CD14 range in healthy blood donors:
1.79-3.68 µg/ml, n=10

Interassay variation coefficient:
9.8-11.8 depending upon concentration

Intraassay variation coefficient:
4.9%, n=10 serum samples

Effective range: 5-50 ng/ml



Assay Procedure

Let all reagents reach room temperature and mix thoroughly

1. **Samples**
Add 100 µl of standards (50, 25, 12.5, 5.25, 3.12 ng/ml = vials a-e) or diluted samples in duplicate into the corresponding wells and incubate for one hour at room temperature with shaking.

2. Wash three times with Wash Buffer (A).

3. **Detection Antibody**
Add 100 µl detection antibody (vial 2) to each well and incubate at room temperature for 1 hour with shaking.

4. Wash three times with Wash Buffer (A).

5. **Substrate**
Add 100 µl Substrate solution (vial 9) to each well. Incubate 13 ± 1 minutes at room temperature without shaking.

6. **Stopping**
Add 100 µl Stopping solution (vial 8) to each well. Tap plate gently to mix.

7. Read absorbance of wells at 450 nm (reference wavelength 620).

8. Calculate the CD14 concentration
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.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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