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# CD14
# Human CD14 Molecule ELISA Kit

**IMPORTANT:**

**NEW INSTRUCTION MANUAL**
**Review prior to kit use.**

**Catalog No:** CKH114

**Size:** 1 x 96 tests

**Introduction**

The human CD14 kit has been developed for the quantitative measurement of natural and recombinant human CD14 in serum, plasma and culture medium. The human CD14 Kit is a solid phase sandwich Enzyme Linked-Immuno-sorbent Assay (ELISA). A mixture of two monoclonal antibodies specific for human CD14 is coated on the wells of the precoated plate. In the first step, the precoated wells 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 is removed by washing. Then a POD-labelled monoclonal antibody specific for CD14 is incubated. Detection step includes TMB as chromogen. The enzyme reaction is stopped by the addition of stop solution (0.25 M sulphuric acid) 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 human CD14 concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve.

**Reagents and materials supplied with the kit:**

| | | |
|---|---|---|
| **1** | **Precoated ELISA plate** | **1 plate** |
| **Vial 2** | **Detection antibody (POD-labelled monoclonal antibody to human CD14) "Ready for use"** | **1 vial** |
| **Vial 3** | **Human CD14-standard (recombinant human CD14)** | **1 vial** |
| **Vial 4** | **Human reference serum – human CD14 content: 2.7 ± 0.5** | **1 vial** |
| **Vial 5** | **PBS** | **2 tablets** |
| **Vial 6** | **Dilution buffer** | **1 vial** |
| **Vial 7** | **Tween 20** | **1 vial** |
| **Vial 8** | **Stop solution "Ready for use"** | **1 vial** |
| **Vial 9** | **Substrate solution "Ready for use"** | **1 vial** |

**Storage Notes:**

- Store at 2-4°C for short time periods.
- Vials 3 and 4 are lyophilized and can be stored long term at -20°C or -80°C.
- The kit is stable for a few days at room temperature and for 3 days at 37°C.

**Material required but not provided:**

- Orbital shaker
- Micro plate reader for measurement absorbance at 450 nm/620
- Precision pipettes with disposable tips
- 10-1,000 µl adjustable multi-well pipette

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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 0.05 % Tween 20 (100 µl, vial 7)
- Prepared Wash Buffer is stable for 4 weeks at 2-4$^{o}$C.

**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 4$^{o}$C for one to two weeks. **Note:** Use buffer for assay only at room temperature.

**D. Reference Serum**

- Add 10 µl distilled water to Reference serum (Vial 4).
- Dilute with 990 µl Dilution buffer (C). Use 100 µl/well.

**E. CD14-standard**

- Prepare just before use.
- Add 30 µl distilled water to vial 3
- Add then entire content of vial 3 into a new vial to be called 'vial 0'.
- Add 970 µl Dilution buffer (C) and mix carefully.
- Now add 50 µl of vial 0 into a new vial to be called 'vial a'.
- Add 450 µl Dilution buffer (C) to vial a.
- Vial a has a 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 |

Store the standard at -20$^{o}$C.

**D. Detection Antibody**

- Ready to use.
- Mix carefully.

**E. Stop Solution**

- Ready to use.
- Mix carefully.

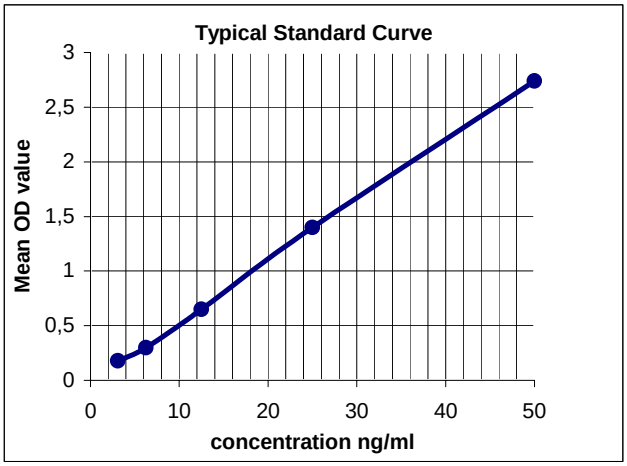
**F. Substrate Solution**

- Ready to use.
- Mix carefully.

**Preparation of samples**

Serum, plasma and other CD14 containing solutions are suitable for use in the test. With coagulation inhibitor citrate the CD14 content is lower than with EDTA or heparin. Samples containing a visible precipitate must be clarified prior to use in the assay. Lipemic and hemolysed probes are not possible. Samples should be frozen at -20°C for a long term storage. Depending on the concentration of CD14 in the samples, these have to be diluted with dilution buffer. For normal serum samples a dilution of 1:200 is recommended.

Normal CD14 range in healthy blood donors: (1.79-3.68 µg/ml) n=10
Interassay variation coefficient: 9.8% to 11.8% depending on concentration
Intraassay variation coefficient: 4.9%, n=10 serum samples
Effective range: 5-50 ng/ml
Cross-reactivity: unknown




**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 1 hour at room temperature with orbital shaker.

2. **Wash** (3x) 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 orbital shaker.

4. **Wash** (3x) with Wash Buffer (A).

5. **Substrate**
   - Add 100 µl Substrate Solution (Vial 9) to each well. Incubate 14 ± 1 minute at room temperature without shaking.

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

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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 standards (a-e) (y-axis) and the CD14 concentration (x-axis).
   - Calculate the CD14 concentration of samples from the standard curve and multiply with dilution factor.



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