biometec
GESELLSCHAFT FÜR BIOMEDIZINISCHE TECHNOLOGIEN mbH

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Recommendations for Use

Please note: New instruction sheet

Cat. No 041 Lot: 011108	**Enzyme Immunoassay for Determination of soluble human CD14 with matched antibody pairs**

Test components for 1 plate

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 (2.7 ± 0.5, lyophilized)**	**1 vial**
Vial 5	**PBS**	**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**

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.

Material required but not provided:

- orbital shaker
- micro plate reader for measurement absorbance at 450 nm/620
- precision pipettes with disposable tips
- 10-1000 µl adjustable multiwell pipette

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 0.05% Tween 20 (100 µl, **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 50µl Tween 20 from **vial 7**. This buffer is 1-2 weeks stable at 4°C. Attention! Use buffer for assay at **room temperature.**

D **Reference serum:** For reconstitution of lyophilized reference serum adds 10 µl distilled water and than dilute with 990µl Dilution buffer (**C**). For testing use 100 µl /well.

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) with 970µl Dilution buffer (**C**) and mix carefully. Now use 50µl of vial 0 and add 450µl Dilution buffer (**C**).This represents = vial a with CD14 concentration of 50ng/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.**

Mix vials 2, 8 and 9 ("Ready for use") carefully before use!!!

PRINCIPLE OF TEST

The Human CD14 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 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 is obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The humanCD14 concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve.

PREPARATION OF SAMPLES

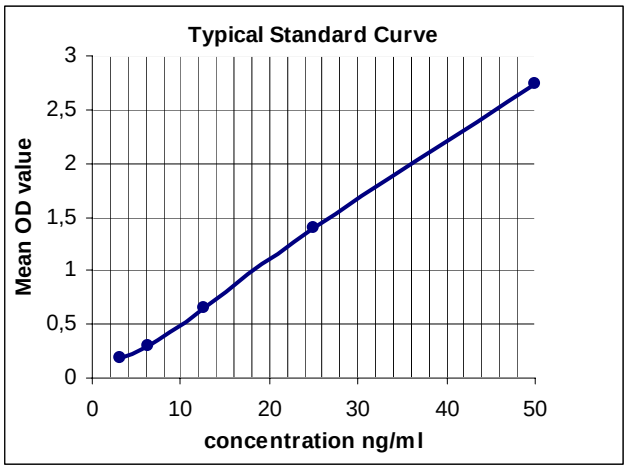
Serum, plasma and other CD14 containing solutions are suitable for use in the test. With coagulation inhibitor citrate the CD14 content is lower then with EDTA or heparin. Samples containing a visible precipitate must be clarified prior to use in the assay. Lipemic and haemolysed probes are not possible.

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 CHARACTERISTIC

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



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 14 ± 1 min at room temperature without shaking.

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)

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.