



## **CD14, Soluble (human) Detection Set [For ELISA Application]**

*Manufactured by Biometec.*

### **ALX-850-302-KI01**

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**For laboratory use only. Not for human or diagnostic use.**

**Test components for 1 plate**

<b>Vial 1</b>	<b>coating antibody (2 monoclonal antibodies to human CD14):</b>	<b>1 vial</b>
<b>Vial 2</b>	<b>detecting antibody (POD-labelled monoclonal antibody to human CD14)</b>	<b>1 vial</b>
<b>Vial 3</b>	<b>CD 14-standard (recombinant human CD14, lyophilized)</b>	<b>1 vial</b>
<b>Vial 4</b>	<b>Blocking reagent</b>	<b>1 vial</b>
<b>Vial 5</b>	<b>Substrate solution A</b>	<b>1 vial</b>
<b>Vial 6</b>	<b>Substrate solution B</b>	<b>1 vial</b>
<b>Vial 7</b>	<b>Adsorption Buffer</b>	<b>1 Capsule</b>
<b>Vial 8</b>	<b>PBS</b>	<b>2 Tablets</b>
<b>Vial 9</b>	<b>Dilution Buffer</b>	<b>1 vial</b>
<b>Vial 10</b>	<b>Tween 20</b>	<b>1 vial</b>
<b>Vial 11</b>	<b>Stopping solution 2 N H<sub>2</sub>SO<sub>4</sub></b>	<b>1 vial</b>
<b>Vial 12</b>	<b>Reference serum (CD14 content 2.7±0.5 µg/ml)</b>	<b>1 vial</b>

Vial 1-2 are stabilised with 0.01 % Thimerosal, vial 3 and 12 are lyophilized  
 Short time store at 2-8°C, Long time storage of vials 1-3 and 12 at -20°C or -80°C  
 The test kit is stable for some days at room temperature and also 3 days at 37°C

**Material required but not provided:**

- ELISA-Plate (NUNC-Maxisorp, F-Form) or Strips
- orbital shaker
- micro plate reader for measurement absorbance at 450 nm/620
- precision pipettes with disposable tips
- 50-200 µl adjustable multiwell pipettes
- Thimerosal

**Preparation of reagents (recommendations for 1 plate)**

- A Wash Buffer:** PBS/ Tween 0.05%/Thimerosal 0.01%:  
 Dissolve 1 Tablet Phosphate buffered saline (PBS, **vial 8**) in 200 ml distilled water and add 0.05 % Tween 20 (100 µl, **vial 9**) and add 0.01 % Thimerosal
- B Adsorption Buffer:** Dissolve **content** of 1 capsule (**vial7**) in 25 ml distilled water. Remove the empty capsule.  
**Alternatively:** 0.2M Carbonate buffer pH 9.3-9.7
- C PBS:** Dilute 1 Tablet of **vial 8** in 200 ml distilled water
- D Dilution buffer:** Dissolve content of **vial 9** with 50 ml PBS (Buffer **C**) and add 50µl Tween 20 from **vial 10**. This buffer is 1-2 weeks stable at 4°C. Attention! Use buffer for assay at **room temperature**.

**ALL BUFFERS ARE RECOMMENDATIONS!**

- E Blocking Reagent:** Add content of the **vial 4** to 40ml PBS (Buffer **C**). Prepare just before use. Store remaining blocking reagent after reconstitution at -20°C
- F Substrate:** Mix carefully 5 ml substrate Solution A (**vial 5**) and 5 ml Solution B (**vial 6**) per plate. Prepare just before use
- G Coating antibody:** Dissolve content of **vial 1** with 10 ml Adsorption Buffer (**B**) Prepare just before use.
- H Detecting antibody:** Dissolve content of **vial 2** with 10 ml blocking reagent (**E**). Prepare just before use.
- I Reference serum:** For reconstitution of lyophilized reference serum adds 10 µl distilled water and than dilute with 990µl Dilution buffer (**D**). For testing use 100 µl /well
- J CD14-standard:** Add 30µl distilled water to the **vial 3** for reconstitution and than 970µl Dilution buffer (**D**). Now use 50µl of this vial and add 450µl Dilution buffer (**D**). This is **vial a** with CD14 concentration of 50ng/ml.  
 For standard curve prepare and use **vial b –e**

No	CD14-Standard dilution µl	Dilution buffer (D)	Concentration ng/ml
<b>vial a</b>			50
<b>vial b</b>	250 µl of <b>vial a</b>	250 µl	25
<b>vial c</b>	250 µl of <b>vial b</b>	250 µl	12.5
<b>vial d</b>	250 µl of <b>vial c</b>	250 µl	6.25
<b>vial e</b>	250 µl of <b>vial d</b>	250 µl	3.125

Prepare just before use. **Store the standard at -20°C.**

- K Thimerosal:** Pipette 10 µl of stock solution (0.2%ig) to 200ml wash buffer **A**

## **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 for coating. After blocking of free binding sites at the well the antigen (standard or sample) will be incubated. 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 sCD14 is incubated. Revelation step includes TMB as chromogen. The enzyme reaction is stopped by the addition of 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.

## **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 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**

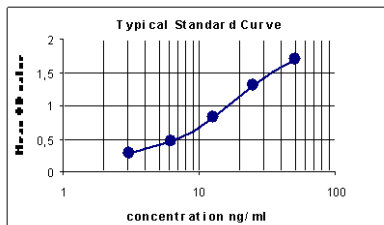
**Normal CD14 range** in healthy blood donors: (1.79-3.68 µg/ml) n= 10

**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. Coating**

Pipette 100 µl coating antibody (G) to each well and incubate over night at 4°C. Cover the plate.

2. 1 x washing with Wash Buffer (A). Remove the Wash Buffer carefully after each wash.

### **3. Blocking**

Add 200 µl Blocking reagents (E) to each well and incubate at room temperature for 30 minutes at orbital shaker (300 U/min)

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

### **5. 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.

6. 3 x washing with Wash Buffer (A).

### **7. Detecting antibody**

Add 100 µl detecting antibody (H) to each well and incubate at room temperature for 1 hour at shaker.

8. 3 x washing with Wash Buffer (A).

### **9. Substrate**

Add 100 µl Substrate solution (F) to each well. Incubate 7 ±2 min at room temperature without shaking.

### **10. Stopping**

Add 100 µl stopping solution (vial 11) to each well. Tape plate gently to mix

11. Read absorbance of wells at 450 nm (reference wave length 620)

### **12. 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 (b-f) (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.

