

**KAMIYA BIOMEDICAL COMPANY**

# Mouse CD14 quantification ELISA

**For the quantitative determination of natural and recombinant mouse  
CD14 in serum, plasma, other body fluids and culture medium**

**Cat. No. KT-1001**

**For Research Use Only. Not for use in diagnostic procedures.**

## Product Information

### Enzyme Immunoassay for quantification of **soluble mouse CD14** Cat. No. KT-1001

## INTENDED USE

The kit is a sandwich enzyme immunoassay for the *in vitro* quantitative measurement of natural and recombinant mouse CD14 in serum, plasma and culture medium. For research use only. Not for use in diagnostic procedures.

## COMPONENTS

No.	Reagents	Quantity
<b>1</b>	Precoated modules, 96-well strip plate	1 plate
<b>Vial 2</b>	Detecting antibody (HRP-labelled polyclonal antibodies to mouse CD14)	1 vial
<b>Vial 3</b>	CD14-standard (recombinant mouse CD14, lyophilized)	1 vial
<b>Vial 4</b>	Reference serum (lyophilized)	1 vial
<b>Vial 5</b>	PBS (Phosphate buffered salt solution)	2 tab.
<b>Vial 6</b>	Dilution Buffer	1 vial
<b>Vial 7</b>	Tween 20	1 vial
<b>Vial 8</b>	Stopping solution "Ready for use"	1 vial
<b>Vial 9</b>	Substrate solution "Ready for use"	1 vial
<b>Vial 10</b>	Dilution buffer for Detecting antibody	1 vial

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Orbital shaker.
2. Micro plate reader for measurement absorbance at 450/620nm.
3. Precision pipettes with disposable tips.
4. 10-1000 µl adjustable multiwell pipette.

## STORAGE

All the reagents should be kept according to the labels on vials. 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.

## PRINCIPLE

The mouse CD14 kit has been developed for the quantitative measurement of natural and recombinant mouse CD14 in serum, plasma and culture medium.

The sCD14 Kit is a solid phase sandwich Enzyme-Linked-Immunosorbent-Assay (ELISA). A mixture of monoclonal antibodies specific for mouse sCD14 is coated at modules. In the first step the precoated

modules will be incubated with the antigen (standard or sample) together with a POD-labelled antibody specific for mouse sCD14. During this incubation, mouse CD14 is captured by solid bound antibody. Unbound material present in the sample is removed by washing. Revelation step includes TMB as chromogen. The enzyme reaction is stopped by the addition of sulphuric acid (0.25M) 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 mouseCD14 concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve.

*The dilution step of sample with second antibody is incorporated in standard curve.*

## REAGENT PREPARATION

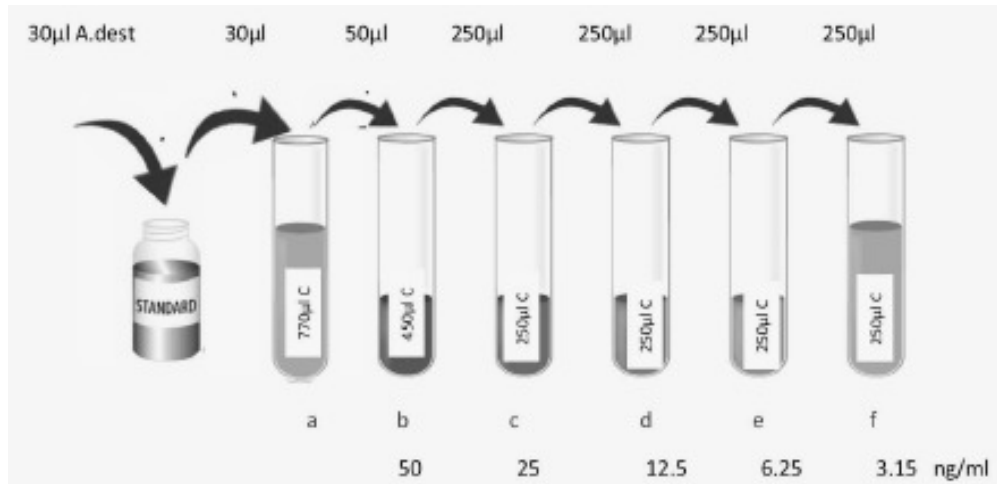


**Note:** Use all reagents for assay at room temperature.

- A Wash buffer:** Dissolve 1 tablet PBS (**vial 5**) in 200 ml distilled water and add 100µl Tween 20 (**vial 7**), store at room temperature. Prepared wash buffer is stable for 4 weeks at refrigerator.
- B PBS:** (Phosphate balanced salt solution) Dilute 1 tablet of **vial 5** in 200 ml distilled water. Store and use at room temperature.
- C Sample dilution buffer:** Dissolve content of **vial 6** with 50 ml PBS (Buffer **C**) and add 50µl Tween 20 from **vial 7**. Use buffer at room temperature. This buffer is 1-2 weeks stable at 4°C.
- D Detecting ab dilution buffer:** Add whole content of the **vial 10** to 13ml PBS (Buffer **B**). Prepare just before use. Store remaining buffer after reconstitution at -20°C.
- E Detecting antibody:** **Firstly** add 500 µl dilution buffer for detecting antibody (**D**) to **vial 2** for solubility (=0.23µg/ml IgG) , mix carefully and **secondly** add the **250µl** of this **vial 2** in a new vial containing 12 ml of **D**. Prepare just before use.
- F Reference mouse serum lyophilized:** Add 10µl distilled water to **vial 4** for solubility and secondly dilute the whole content of **vial 4** with 2990 µl dilution buffer for samples (**C**) in a new vial. Pipette 50µl/well. This represents a dilution of 1:300. The mCD14 content of this reference serum is 4.3 ± 2.2µg/ml.
- G Mouse CD14-standard lyophilized:** **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 with 770µl sample dilution buffer (**C**) and mix carefully. This is **vial a**. For standard curve prepare vial **b-f**. Prepare just before use.

**Reconstituted standard can be stored for 1 week at refrigerator or -80°C for 2-3 weeks.**

No.	Mouse CD14 µl	Dilution buffer ( <b>C</b> )	Concentration ng/ml
<b>Vial b</b>	50 µl of vial <b>a</b>	450 µl	50
<b>Vial c</b>	250 µl of vial <b>b</b>	250 µl	25
<b>Vial d</b>	250 µl of vial <b>c</b>	250 µl	12.5
<b>Vial e</b>	250 µl of vial <b>d</b>	250 µl	6.25
<b>Vial f</b>	250 µl of vial <b>e</b>	250 µl	3.12



## SAMPLE PREPARATION

Kamiya Biomedical Company is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

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 to 1:300 is recommended. The CD14 content of mouse normal serum is 0.3 – 6µg/ml. After infection the CD14 content can be 10-100 times higher.

## ASSAY CHARACTERISTIC

**Normal CD14 range** in healthy mice: (0.3 - 6µg/ml) n= 10

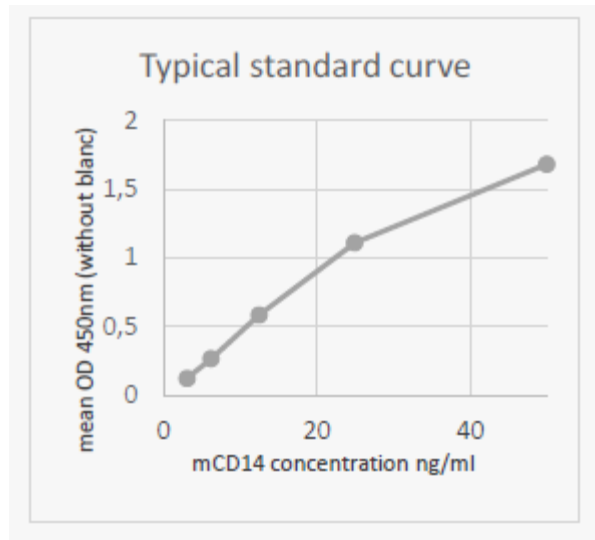
**Interassay** variation coefficient: 9.8% till 17.8 depending of Concentration.

**Intraassay** variation coefficient: 6.9%, n=10 serum samples.

**Effective range:** 5 -50 ng/ml

**Cross reaction:** no reaction with human, rabbit, horse, pork, bovine or rat CD14.

**Stability:** Test kit is stable 3 days at 37°C, 1 week at room temperature, 1 year at refrigerator if standard and reference are stored at - 20°C.



## ASSAY PROCEDURE

Let all reagents reach room temperature and mix thoroughly

- 1. Samples and detecting antibody**  
Add 50 µl of standards (G) vial b-f (50, 25, 12.5, 6.25, 3.12 ng/ml), reference (F) or diluted samples in duplicate into the corresponding wells **as well as** 50µl detecting antibody (E). Incubate for 1.5 hours at room temperature with shaking.
- 2.** 3 x washing with 250µl Wash Buffer/well (A). Remove the Wash Buffer carefully after each wash.
- 3. Substrate**  
Add 100 µl Substrate solution (vial 9) to each well. Incubate  $14 \pm 1$  min at room temperature **without** shaking **in the dark** up to strong colour change to blue is visible.
- 4. Stopping**  
Add 100 µl stopping solution (vial 8) to each well. Tape plate gently to mix; now colour is yellow.
- 5.** Read absorbance of wells at 450 nm (reference wave length 620)

## CALCULATION mCD14-CONCENTRATION

Calculate the mean optical density (OD) of standard duplicates, reference serum and the samples. Design a standard curve by plotting the OD means of standards b-f) (y-axis) and the CD14 concentration (x-axis). Calculate the mCD14 concentration of samples from the standard curve and multiply with dilution factor.

**FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.**

## KAMIYA BIOMEDICAL COMPANY

12779 Gateway Drive, Seattle, WA 98168  
Tel: (206) 575-8068 Fax: (206) 575-8094  
Email: LifeScience@k-assay.com  
[www.k-assay.com](http://www.k-assay.com)