biometec
COMPANY FOR BIOMEDICAL TECHNOLOGIES INC.

Walther-Rathenau-Str. 49a, D-17489 Greifswald,
Tel. ++493834515620, Fax ++493834515622, e-mail: witt@biometec.de, www.biometec.de

Recommendations for Use

Please note: New lot and new reference serum

Cat. No 042 **Lot 140414**	**"One step" Enzyme Immunoassay for quantification of** soluble mouse CD14

TEST COMPONENTS

1	precoated ELISA module	1 plate
Vial 2	detecting antibody (HRP-labelled polyclonal antibodies to mouse CD14)	1 vial
Vial 3	CD14-standard (recombinant mouse CD14, lyophilized)	1 vial
Vial 4	Reference serum (lyophilized)	1 vial
Vial 5	PBS	2 tab.
Vial 6	Dilution buffer for samples	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
Vial 10	Dilution buffer for Detecting antibody	1 vial

Vials 2 is stabilised with 0.01 % Thimerosal; vial 3 and 4 are lyophilized

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 450/620 nm
- precision pipettes with disposable tips
- 10-1000 µl adjustable multi-well pipette

PREPARATION OF REAGENTS: Attention! Use all reagents for assay at room temperature.

A **Wash Buffer**: Dissolve 1 tablet PBS (**vial 5**) in 200 ml distilled water-add 100 µl 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 **vial10** 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 **C**	Concentration ng/ml
vial b	50 µl of a	450 µl	50
vial c	250 µl of vial b	250 µl	25
vial d	250 µl of vial c	250 µl	12.5
vial e	250 µl of vial d	250 µl	6.25
vial f	250 µl of vial e	250 µl	3.12

PRINCIPLE OF THE TEST

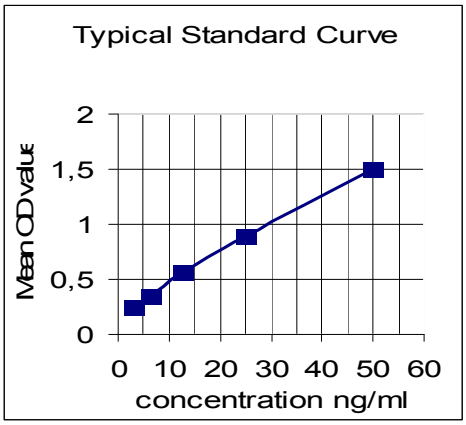
The mouse CD14 kit has been developed for the quantitative measurement of natural and recombinant mouse CD14 in serum, plasma and culture medium. The sCD14 Kit is a solid phase sandwich Enzyme-Linked-Immunosorbent-Assay (ELISA). A mixture of monoclonal antibodies specific for mouse sCD14 is coated at 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 (O.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.**

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 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 minus 20°C.



ASSAY PROCEDURE FOR "ONE STEP" ASSAY

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 **(vial 9)** to each well. Incubate 14 ± 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).

6. **Calculate 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 (a-f) (y-axis) and the CD14 concentration (x-axis). Calculate the mCD14 concentration of samples from the standard curve and multiply with dilution factor.

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