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CD14
Mouse CD14 Antigen ELISA Kit

IMPORTANT:

NEW INSTRUCTION MANUAL
Review prior to kit use.

Catalog No: CKM034

Size: 1 x 96 tests

Introduction:

The mouse CD14 kit has been developed for the quantitative measurement of natural and recombinant mouse CD14 in serum, plasma and culture medium. The mouse CD14 Kit is a solid phase sandwich Enzyme Linked-Immuno-sorbent Assay (ELISA). A mixture of monoclonal antibodies specific for mouse CD14 is used on the well of the precoated plate. In the first step, the precoated wells will be incubated with the antigen (standard or sample) together with a POD-labelled antibody specific for mouse CD14. During this incubation, mouse CD14 is captured by solid bound antibody. Unbound material present in the sample is removed by washing. 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 mouse CD14 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 the standard curve.

Test Components supplied with the kit:

1	**Precoated ELISA plate**	**1 plate**
Vial 2	**Detection antibody (POD-labelled polyclonal antibodies to mouse CD14)**	**1 vial**
Vial 3	**Mouse CD14-standard (recombinant mouse CD14, lyophilized)**	**1 vial**
Vial 4	**Mouse reference serum - mouse CD14 content: 2.7 ± 0.5 µg/ml**	**1 vial**
Vial 5	**PBS**	**2 tablets**
Vial 6	**Sample 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**
Vial 10	**Detection antibody dilution buffer**	**1 vial**

Storage Notes:

- Vial 2 is stabilized with 0.01% thimerosal. Precaution: Thiomersal is a poisonous and hazardous substance which should be handled by trained staff only.
- 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.

Materials required but not provided:

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

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Preparation of reagents:

A. Wash Buffer
PBS/Tween 0.05%
Dissolve 1 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°C.

B. PBS
Dilute 1 Tablet of Vial 5 in 200 ml distilled water.
Store and use at room temperature.

C. Sample Dilution Buffer
Add contents of Vial 6 to 50 ml PBS (Buffer C).
Add 50 µl Tween 20 (Vial 7).
Use buffer at room temperature. This buffer is stable for 1-2 weeks at 2-4°C.

D. Detection Antibody Dilution Buffer
Prepare just before use.
Add contents of Detection Antibody Dilution Buffer (Vial 10) to 10 ml PBS (Buffer B).
After reconstitution store remaining buffer at -20°C.

E. Detection Antibody
Prepare just before use.
Add 500 µl Detection Antibody Dilution Buffer (D) to Vial 2.
Mix carefully.
Dissolve 250 µl of this Vial 2 in 8 ml Detection Antibody Dilution Buffer (D).

F. Reference Serum
Add 10 µl distilled water to Vial 4.
Dilute the whole content of Vial 4 with 1490 µl sample dilution buffer in a new vial (C).
This is a dilution of 1:150. Use 50 µl/well.
The reference serum contains 2.7 ± 0.5 µg/ml CD14.

G. Mouse CD14 Standard
Prepare just before use.
Pipette 30 µl distilled water to Vial 3 for reconstitution.
Add the entire contents of Vial 3 into a new vial to be called 'vial a'.
Add 770 µl Sample Dilution Buffer (C) to vial a and mix carefully.
For standard curve prepare vials b-f.

	Mouse CD14 µl	Dilution Buffer C	Concentration ng/ml
vial b	50 µl of vial 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

Store the standard at -20°C or -80°C.

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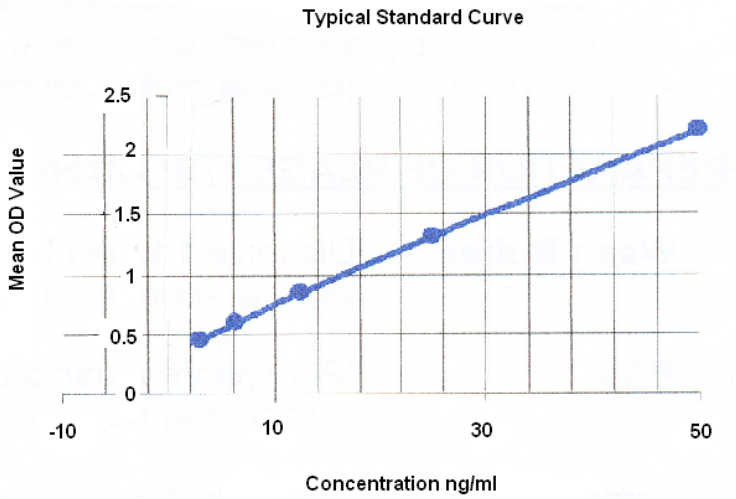
Preparation of samples

Serum, plasma and other mouse 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 hemolytic probes are not possible. Samples should be frozen at -20°C for long term storage. Depending on the concentration of mouse CD14 in the samples, these have to be diluted with dilution buffer. For normal serum samples a dilution of 1:100 – 1:150 is recommended. The CD14 content of normal mouse serum is 0.3 – 6 µg/ml. After infection the CD14 content can be 10 to 100 times higher.

Assay Characteristics

Normal CD14 range in healthy mice: (0.3 - 6 µg/ml). n=10.
Interassay variation coefficient: 9.8% to 17.8% depending on the concentration
Intrassay variation coefficient: 6.9%, n= 10 serum samples
Effective range: 5 - 50 ng/ml
Cross reaction: No cross-reactivity with human, rabbit, horse, pig, cow, or rat CD14.
Stability: Test kit is stable for 3 days at 37°C, 1 week at room temperature, and 1 year at 2-4 °C if standard and reference are stored at -20 °C.



Assay Procedure

Let all reagents reach room temperature and mix thoroughly.

1. **Samples and Detection antibody**
   Add 50 µl of standards (**G**) (50, 25, 12.5, 6.25, 3.12 ng/ml = vials b-f), reference (**F**), or diluted samples in duplicate into the corresponding wells of the precoated plates as well as 50 µl detection antibody (**E**). Incubate for 1.5 hours at room temperature with orbital shaker.

2. **Wash** 3X with 250 µl/well Wash Buffer (**A**).
   Remove Wash Buffer carefully after each wash.

3. **Substrate**
   Add 100 µl Substrate solutions (**Vial 9**) to each well.
   Incubate 14 ± 1 min in the dark at room temperature without shaking until strong color change to blue is visible.

4. **Stopping**
   Add 100 µl stop solution (**Vial 8**) to each well.
   Tap plate gently to mix.
   Color is now yellow.

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

6. **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-f) (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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