CD14, Soluble (mouse) ELISA Kit

Manufactured by Biometec.

ALX-850-303/1-KI01

96 wells (~80 tests)

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NORTH AMERICA

AXXORA, LLC
6181 Cornerstone Court East
Suite 103
San Diego, CA 92121-4727
Phone: (858) 550-8828
Fax: (858) 550-8825
E-mail: axxora-usa@axxora.com

SWITZERLAND/REST OF THE WORLD

ALEXIS CORPORATION
Industriestrasse 17, Postfach
CH-4415 Lausen / Switzerland
Phone: +41 61 926 89 89
Fax: +41 61 926 89 79
E-mail: alexis-ch@alexis-corp.com

GERMANY

AXXORA DEUTSCHLAND GmbH
Marie-Curie-Strasse 8
DE-79539 Lörrach
Phone: (07621) 5500 522
Fax: (07621) 5500 523
E-mail: axxora-de@axxora.com

UK & IRELAND

AXXORA (UK) LTD.
P.O. Box 6757
Bingham, Nottingham NG13 8LS
Phone: +44 1949 836111
Fax: +44 1949 836222
E-mail: axxora-uk@axxora.com

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

TEST COMPONENTS

1	**precoated ELISA module**	**1 plate**
Vial 2	**detecting antibody (POD-labelled polyclonal antibodies to mouse CD14)**	**1 vial**
Vial 3	**CD14-standard (recombinant mouse CD14, lyophilized)**	**1 vial**
Vial 4	**Reference serum (2.7 ± 0.5, lyophilized)**	**1 vial**
Vial 5	**PBS**	**2 tab.**
Vial 6	**Sample 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**
Vial 10	**Detecting ab dilution buffer**	**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 0.05 % Tween 20 (100 µl of **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 content of the **vial10** to 10ml PBS (Buffer **B**). Prepare just before use. Store remaining buffer after reconstitution at -20°C

E **Detecting antibody**: Add 500 µl detecting ab dilution buffer (**D**) to **vial 2**, mix carefully and than dissolve 250µl of this **vial 2** in 8 ml detecting ab dilution buffer (**D**). Prepare just before use.

F **Reference mouse serum lyophilized:** Add 10µl distilled water to **vial 4** for solubility and than dilute the whole content with 1490 µl sample dilution buffer (**C**). Pipette 50µl/well. This represents a dilution of 1:150. The mCD14 content of this reference serum is 3.4±0.6 µg/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.

Store standard at -20 or -80°C

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

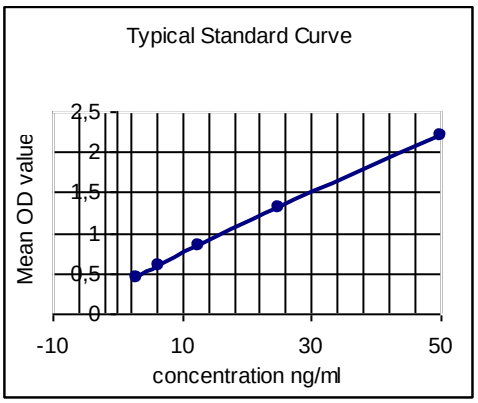
The mouse CD14 kit has been developed for the quantitative measurement of natural and recombinant mouse CD14 in serum, plasma and culture medium. The sCD14 Kit is a solid phase sandwich Enzyme-Linked-Immunosorbent-Assay (ELISA). A mixture of monoclonal antibodies specific for mouse sCD14 is coated at 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 1:100 to 1:150 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 a-e (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.

6. 3 x washing with 250µl Wash Buffer/well **(A).** Remove the Wash Buffer carefully after each wash.

7. **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.

8. **Stopping**
   Add 100 µl stopping solution **(vial 8)** to each well. Tape plate gently to mix; now colour is yellow

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

10. **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.

REF: Dessing, M.C. et al. 2007: Amer.J. Respirat and critical Care Med 175, pp 604-611
Gerhold, K. et al. 2006, J. of Allergy and Clinical Immunology 118/3 pp 666-673