CD14, Soluble (mouse) Detection Set [For ELISA Application]

Manufactured by Biometec.

ALX-850-303-KI01

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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 FOR ONE 96 WELL PLATE

Vial 1	**coating monoclonal (mix of monoclonal antibodies to mouse CD14)**	**1 vial**
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	**blocking reagent**	**1 vial**
Vial 5	**substrate (ready for use)**	**1 vial**
Vial 7	**adsorption buffer**	**1 capsule**
Vial 8	**PBS**	**2 tablets**
Vial 9	**dilution buffer**	**1 vial**
Vial10	**tween20**	**1 vial**
Vial11	**reference serum (lyophilized)**	**1 vial**
Vial12	**stopping solution (ready for use)**	**1 vial**

Vials 1-2 are stabilised with 0.01 % Thimerosal; vial 3 and 11 are lyophilized
Short time store at 2-8°C, long time storage of vial 1, 2, 3 and 11 at -20°C or -80°C

MATERIAL REQUIRED BUT NOT PROVIDED:

- **96well ELISA-Plate** (NUNC-Maxisorp, F-Form) or Strips
- orbital shaker
- micro plate reader for measurement absorbance at 450 nm
- precision pipettes with disposable tips
- 50-200 µl adjustable multiwell pipette
- Thimerosal

PREPARATION OF REAGENTS:

A **Wash Buffer**: Dissolve 1 tablet PBS (**vial 8**) in 200 ml distilled water-add 0.05 % Tween 20 (100 µl of vial **10**) and add 0.01 % Thimerosal, store at room temperature

B **Adsorption Buffer**, **pH 9.4**: Dissolve **content** of one capsule (**vial 7**) in 25 ml distilled water. Remove the empty capsule. The pH should be in order without corrections (**Alternatively:** 0.2M Carbonate buffer pH 9.3-9.7

C **PBS**: (Phosphate balanced salt solution) Dilute 1 tablet of vial **8** in 200 ml distilled water. Store and use at room temperature.

D **Dilution buffer:** Dissolve content of **vial 9** with 50 ml PBS (Buffer **C**) and add 50µl Tween 20 from **vial 10**. Use buffer at room temperature. This buffer is 1-2 weeks stable at 4°C.

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 **Coating antibody**: Add content of the **vial 1** to 10 ml Adsorption Buffer (**B**). Prepare just before use.

G **Detecting antibody**: Add 10µl of **vial 2** to 8 ml blocking reagent (**E**). Prepare just before use.

H **Reference mouse serum lyophilized:** Add 10µl water to **vial 11** for solubility and than dilute the whole content with 1490 µl dilution buffer (**D**). Pipette 50µl/well. This represents a dilution of 1:150. The mCD14 content of this reference serum is 3.4±0.6 µg/ml.

I **Mouse CD14-standard lyophilized:** Add 30 µl water to **vial 3** for solubility and than add 770µl Dilution Buffer (**D**) to the **vial 3**. For standard curve prepare vial **a-e.** Prepare just before use.
Store standard at -20 or -80°C

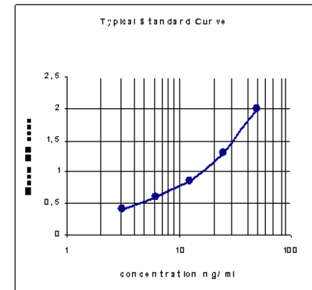
No	Mouse CD14 µl	Dilution buffer **D**	Concentration ng/ml
vial a	50 µl of **vial 3**	450 µl	50
vial b	250 µl of vial a	250 µl	25
vial c	250 µl of vial b	250 µl	12.5
vial d	250 µl of vial c	250 µl	6.25
vial e	250 µl of vial d	250 µl	3.12

Attention! Use all reagents for assay at room temperature. ALL BUFFERS ARE RECOMMENDATIONS!

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 for coating. The antigen and a POD-labelled antibody specific for mouse sCD14 is second incubation step. 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 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, 3 months at refrigerator and more than 1 year if antibodies, 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. **Coating**
   Pipette 100 µl coating antibody **(F)** to each well and incubate over night at 4°C.
   Cover the plate.

2. Add 200µl Wash Buffer **(A).** Remove the Wash Buffer carefully after washing.

3. **Blocking**
   Add 200 µl Blocking reagent **(E)** to each well and incubate at room temperature for 30 minutes at orbital shaker (300 rpm).

4. Remove blocking reagent carefully and wash 3 times with 250µl Wash Buffer/well **(A).** Remove the Wash Buffer carefully after each wash.

5. **Samples and detecting antibody**
   Add 50 µl of standards **(I)** vial a-e (50, 25, 12.5, 6.25, 3.12 ng/ml), reference **(H)** or diluted samples in duplicate into the corresponding wells **as well as** 50µl detecting antibody **(G)**. 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 5)** to each well. Incubate 10 ± 2 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 12)** 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