

Datasheet for ABIN625274  
**CD14 ELISA Kit**



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## Overview

Quantity:	96 tests
Target:	CD14
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	6-6000 pg/mL
Minimum Detection Limit:	6 pg/mL
Application:	ELISA

## Product Details

Purpose:	Human CD14 ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin, BDNF, BLC, CNTF, ENA- 78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, FGF-4, FGF-6, FGF- 7, G-CSF, GDNF, GM-CSF, IFN-gamma, IGFBP-2, IGFBP-3, IGFBP-4, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIF, MIG, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, SDF-1 alpha, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.
Sensitivity:	< 6 pg/mL

## Product Details

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- Characteristics:
- Strip plates and additional reagents allow for use in multiple experiments
  - Quantitative protein detection
  - Establishes normal range
  - The best products for confirmation of antibody array data

- Components:
- Pre-Coated 96-well Strip Microplate
  - Wash Buffer
  - Stop Solution
  - Assay Diluent(s)
  - Lyophilized Standard
  - Biotinylated Detection Antibody
  - Streptavidin-Conjugated HRP
  - TMB One-Step Substrate

- Material not included:
- Distilled or deionized water
  - Precision pipettes to deliver 2  $\mu$ L to 1  $\mu$ L volumes
  - Adjustable 1-25  $\mu$ L pipettes for reagent preparation
  - 100  $\mu$ L and 1 liter graduated cylinders
  - Tubes to prepare standard and sample dilutions
  - Absorbent paper
  - Microplate reader capable of measuring absorbance at 450nm
  - Log-log graph paper or computer and software for ELISA data analysis

## Target Details

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Target: CD14

Alternative Name: CD14 ([CD14 Products](#))

Background: The Human CD14 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human CD14 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human CD14 coated on a 96-well plate. Standards and samples are pipetted into the wells and CD14 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human CD14 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CD14 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.

Gene ID: 929

## Target Details

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UniProt: [P08571](#)

Pathways: [TLR Signaling, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Toll-Like Receptors Cascades](#)

## Application Details

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Application Notes: Recommended Dilution for serum and plasma samples 2,000 - 20,000 fold

Sample Volume: 100  $\mu$ L

Plate: Pre-coated

Protocol:

1. Prepare all reagents, samples and standards as instructed in the manual.
2. Add 100  $\mu$ L of standard or sample to each well.
3. Incubate 2.5 h at RT or O/N at 4 °C.
4. Add 100  $\mu$ L of prepared biotin antibody to each well.
5. Incubate 1 h at RT.
6. Add 100  $\mu$ L of prepared Streptavidin solution to each well.
7. Incubate 45 min at RT.
8. Add 100  $\mu$ L of TMB One-Step Substrate Reagent to each well.
9. Incubate 30 min at RT.
10. Add 50  $\mu$ L of Stop Solution to each well.
11. Read at 450 nm immediately.

Reagent Preparation:

1. Bring all reagents and samples to room temperature (18 - 25 °C) before use.
2. Sample dilution: If your samples need to be diluted, 1x Assay Diluent (Item E) should be used for dilution of serum/plasma/culture supernatants/urine. Suggested dilution for normal serum/plasma: 2,000-20,000 fold\*. For example, add 1  $\mu$ L of serum/plasma into a tube with 99  $\mu$ L 1x Assay Diluent to prepare a 100-fold diluted sample. Mix through and then pipette 6  $\mu$ L of prepared 100-fold diluted sample into a tube with 294  $\mu$ L 1x Assay Diluent to prepare a final 5,000 fold diluted sample. \*Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.
3. Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: Briefly spin the vial of Item C. Add 400  $\mu$ L 1x Assay Diluent (Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 50 ng/mL standard solution. Dissolve the powder thoroughly by a gentle mix. Add 60  $\mu$ L CD14 standard (50 ng/nl) from the vial of Item C, into a tube with 440  $\mu$ L 1x Assay Diluent to prepare a 6,000 pg/mL standard solution. Pipette 400  $\mu$ L 1x Assay Diluent into each tube. Use the 6,000 pg/mL standard solution to produce a dilution series. Mix each tube

thoroughly before the next transfer. 1x Assay Diluent serves as the zero standard (0 pg/mL).  
200 µL 200 µL 200 µL 200 µL 200 µL 60 µL standard (50 ng/mL) + 440 µL 200µl 6,000 2,000  
666.7 222.2 74.07 24.69 8.23 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL  
5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.  
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent and used in step 4 of Part VI Assay Procedure.  
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 400-fold with 1x Assay Diluent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 35 µL of HRP-Streptavidin concentrate into a tube with 14 ml 1x Assay Diluent to prepare a final 400 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

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### Assay Procedure:

1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step
6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step
8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

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### Calculation of Results:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and

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## Application Details

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subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent Human CD14 concentration (pg/mL) 1 10 100 1000 10000 O D =4 50 n m 0.01 0.1 1 10

Sensitivity: The minimum detectable dose of CD14 is typically less than 6 pg/mL.

Recovery: Recovery was determined by spiking human CD14 into normal human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 76.23 70-85 Plasma 73.87 67-80 Cell culture media 73.77 67-81

Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 126.8 127.4 116.7 Range (%) 115-134 120-133 110-121 1:4 Average % of Expected 134.4 90.25 112.8 Range (%) 124-139 78-102 84-130

Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %

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Assay Precision: Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %

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Restrictions: For Research Use only

## Handling

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Handling Advice: Avoid repeated freeze-thaw cycles.

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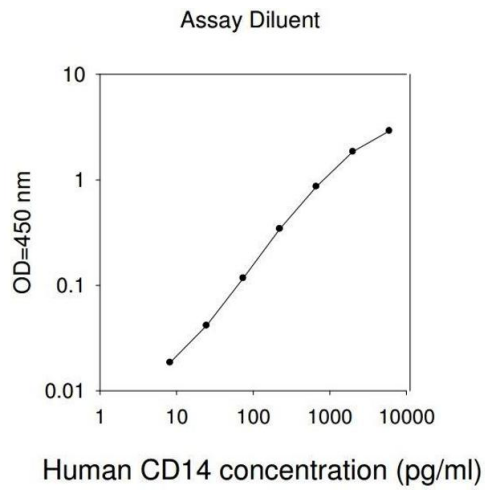
Storage: -20 °C

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Storage Comment: The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.

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Expiry Date: 6 months



ELISA

Image 1.