ab285330 – Mouse IL-6 ELISA Kit

For quantitative measurement of IL-6 in mouse serum, plasma or cell culture supernatants.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285330

Storage and Stability

The entire kit may be stored at 4°C for 6 months or -20°C for 12 months

Materials Supplied

Item	Quantity	Storage Condition
IL-6 mAb coated Microplate	1	- 20°C
Mouse IL-6 Standard (10ng/vial)	2 vials	- 20°C
Biotinylated anti-mouse IL-6 Ab	100 µl	- 20°C
Avidin Biotin-Peroxidase Complex (ABC)	100 µl	- 20°C
Sample diluent buffer	30 ml	- 20°C
Antibody diluent buffer	12 ml	- 20°C
ABC diluent buffer	12 ml	- 20°C
TMB (colourless)	10 ml	- 20°C
TMB stop solution	10 ml	- 20°C
Wash solution	20 ml	- 20°C
Plate sealers	4	- 20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large number of samples in the detection.
- Absorbent paper

Reagent Preparation

Δ Note: Before using the kit, spin tubes and bring down all components to the bottom of tubes. The assay can also be done at RT. However, we recommend doing it at 37 °C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37 °C

IL-6 mAb coated plate, 96 wells: The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Washing buffer (1X): Prepare 500 ml of 1X Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25X) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to RT and mix it gently until crystals have completely dissolved.

Preparation of biotinylated anti-mouse IL-6 antibody working solution: Dilute 1:100 with the antibody diluent buffer and mix thoroughly. Prepare 0.1 ml of IL-6 antibody working solution for each well. Solution should be prepared no more than 2 hr, prior to the experiment.

Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: Dilute 1:100 with the ABC dilution buffer and mix thoroughly. Prepare 0.1 ml of ABC working solution for each well. Solution should be prepared no more than 1 hr, prior to the experiment.

Standard Preparation

Δ Note: Two vials of IL-6 standard (10 ng per vial) are included in each kit. Use one vial for each experiment.

1) Prepare 10,000 pg/ml of mouse IL-6 standard solution by adding 1ml of sample diluent buffer into one of the vials.
2) Keep the tube at room temperature for 10 min. and mix thoroughly.
3) Prepare 1000 pg/ml of mouse IL-6 standard solution by adding 0.1 ml of the 10 ng/ml IL-6 Standard solution into 0.9 ml sample diluent buffer and mix thoroughly.
4) Label 6 Eppendorf tubes with 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml & 15.6 pg/ml respectively.
5) Aliquot 0.3 ml of the sample diluent buffer into each tube.
6) Add 0.3 ml of the 1000 pg/ml IL-6 standard solution into 1st tube and mix.
7) Transfer 0.3 ml from 1st tube to 2nd tube and mix.
8) Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Δ Note: The standard solutions are best used within 2 hr. The 10 ng/ml standard solution should be stored at 4 °C for up to 12 hr, or at - 20 °C for up to 48 hr. Avoid repeated freeze-thaw cycles.

Sample Preparation

Δ Note: Store samples on ice if they will be tested within 2 hours (freshly prepared samples yield the best results). For long-term storage, aliquot and freeze at -20°C (≤ 2 weeks) or -80°C (≤ 1 months). Avoid repeated freeze-thaw cycles. Bring samples to room temperature before the assay.

1) Centrifuge cell culture supernatants to remove particulates, assay immediately or aliquot and store at -20 °C.
2) Allow the serum to clot in a serum separator tube (~ 4 hr) at RT.
3) Centrifuge at approximately 1000 X g for 15 min.
4) Analyze the serum immediately or aliquot and store frozen at -20 °C. Collect plasma using heparin, citrate or EDTA as an anticoagulant.
5) Centrifuge for 15 min at 1000 x g within 30 min of collection.
6) Analyze immediately or aliquot and store frozen at -20 °C.

Sample dilution guidelines:

- The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.
- Dilute the sample using the provided diluent buffer. The sample must be well mixed with the diluents buffer.
- The following is a guideline for sample dilution. Several trials may be necessary in practice.
- For high target protein concentration (10-100 ng/ml): dilute 1:100.
- For medium target protein concentration (1-10 ng/ml): dilute 1:10.
- For low target protein concentration (15.6-1000 pg/ml): dilute 1:2.
- For very low target protein concentration (≤ 15.6 pg/ml). No dilution necessary or dilute 1:2.

Δ Note: Store samples to be assayed within 24 hr at 2-8 °C. For long-term storage, aliquot and freeze samples at -20 °C. Avoid repeated freeze-thaw cycles

Assay Protocol

Δ Note: The ABC working solution and TMB color developing agent must be kept warm at 37 °C for 30 min. before use. When diluting samples and reagents, they must be mixed completely and evenly. Don't let 96-well plate dry, as it will inactivate active components on plate.

1) Aliquot 0.1 ml per well of the 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml and 15.6 pg/ml mouse IL-6 standard solutions into the precoated 96-well plate.
2) Add 0.1 ml of the Sample diluent buffer into the control well (Zero well).
3) Add 0.1 ml of each properly diluted sample of mouse cell culture supernatants, serum or plasma to each empty well. See "Sample Dilution Guideline" for details.

Δ Notes:

a. We recommend that each mouse IL-6 standard solution and each sample is measured in duplicates.
b. We recommend doing a pilot experiment using standards and a small number of samples to inspect the validity of experiment operation and the appropriateness of sample dilution proportion.

4) Seal the plate with the cover and incubate at 37 °C for 90 min or 120 min at RT.
5) Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.
6) Add 0.1 ml of biotinylated anti-mouse IL-6 antibody working solution into each well and incubate the plate at 37°C for 60 min or 90 min at RT.
7) Wash plate 3 times with 1X Wash Buffer, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
   Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml 1X Wash Buffer for 1~2 min. Repeat this process two additional times for a total of three washes.

Δ Note: For automated washing, aspirate all wells and wash three times with 1X Wash Buffer. Blot the plate onto paper towels or other absorbent material.)

8) Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37 °C for 30 min or 40 min at RT.
9) Wash plate 5 times with 1X Wash Buffer, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
10) Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37 °C in dark for 15-25 min or 30 min at RT.

Δ Note: For reference only, the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the four most concentrated mouse IL-6 Standard solutions; the other wells show no obvious color.

11) Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.

Measurement

Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

Calculation

1) Relative O.D450 nm = O.D450 nm of each well – O.D450 nm of Zero well.
2) The Standard Curve can be plotted as the relative O.D450 nm of each standard solution (Y) vs. the respective concentration of the standard solution (X).
3) The mouse IL-6 concentration of the samples can be interpolated from the Standard Curve. It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit.

Δ Note: If the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

Technical Support

For all technical or commercial enquiries please go to:
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