ab100712 IL-6 Mouse ELISA Kit

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

For overview, typical data and additional information please visit: www.abcam.com/ab100712

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: www.abcam.com/protocols/the-complete-elisa-guide

Protocol Summary

- Prepare all reagents, samples, and standards as instructed
- Add standard or sample to appropriate wells. Incubate at room temperature.
- Wash and add prepared biotin antibody to each well. Incubate at room temperature.
- Wash and add prepared Streptavidin Solution. Incubate at room temperature.
- Add TMB One-Step Development Solution to each well. Incubate at room temperature
- Add Stop Solution to each well. Read at 450 nm immediately.

Storage and Stability:

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Precautions

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

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Materials Supplied

Item	Quantity	Storage Condition
IL-6 Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer	25 ml	-20°C
5X Assay Diluent	15 ml	-20°C
Biotinylated anti-mouse IL-6	2 vials	-20°C
Recombinant mouse IL-6 Standard	2 vials	-20°C
200X HRP-Streptavidin Concentrate	200 µl	-20°C
TMB One-Step Substrate Reagent	12 ml	-20°C
Stop Solution	8 ml	-20°C

Materials Required, Not Supplied

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

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

1X Assay Diluent: 5X Assay Diluent should be diluted 5-fold with deionized or distilled water before use (can be stored at 4°C for 1 month after preparation).

1X Wash Buffer: If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature, and mix gently until dissolved. Dilute 20 ml of 20X Wash Solution Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Solution. The prepared Wash Solution can be stored at 4°C for 1 month.
1X Biotinylated IL-6 Detection Antibody: Briefly spin the Biotinylated anti-mouse IL-6 vial 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 either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate must be diluted 80-fold with 1X Assay Diluent prior to use in the Assay Procedure.

1X HRP-Streptavidin Concentrate: Briefly centrifuge the 200X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. The 200X HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent.
For example: Briefly centrifuge the vial and pipette up and down to mix gently. Add 60 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent to prepare a 1X HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix well.

Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Standard (recombinant protein) should be stored at -20°C or 80°C (recommended at -80°C) after reconstitution.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Briefly spin the vial of IL-6 Standard. Prepare the 50 ng/mL **Stock Standard** by adding 640 µL 1X Assay Diluent into the vial (see table below).
2. Ensure the powder is thoroughly dissolved by gentle mixing.
3. Label tubes #1 – 7.
4. Prepare **Standard #1** by adding 8 µL of the 50 ng/mL **Stock Standard**, to 658.7 µL of 1X Assay Diluent into tube 1#. Mix thoroughly and gently.
5. Pipette 400 µL of 1X Assay Diluent into remaining tubes.
6. Prepare Standard #2 by adding 200 µL **Standard #1** to tube #2 and mix thoroughly.
7. Prepare Standard #3 by adding 200 µL **Standard #2** to tube #3 and mix thoroughly.
8. Using the table below as a guide, prepare subsequent serial dilutions. Standard #8 contains no protein and is the Blank control.

Standard (tube)#	Volume to dilute	Volume of 1X Assay Diluent	Total Volume (µL)	Starting Concentration pg/mL	Final Concentration pg/mL
1	8	658.7	666.7	50000	600
2	200	400	600	600	200
3	200	400	600	200	66.7
4	200	400	600	66.7	22.2
5	200	400	600	22.2	7.4
6	200	400	600	7.4	2.5
7	200	400	600	2.5	0.82
8 (blank)	0	400	400	0	0

Sample Preparation

- Suggested dilution for normal serum/plasma: 2-fold.
- 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.

Plate preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

1. Add 100 µl of each standard (see Standard Preparation) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
2. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (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 1X Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
3. Add 100 µL of 1X Biotinylated IL-6 Detection Antibody (Reagent Preparation) to each well. Incubate for 1 hour at room temperature with gentle shaking.
4. Discard the solution. Repeat the wash as in step 2.
5. Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 2.
7. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
8. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.
9. Analyze the data as described below.
10. Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average Blank absorbance value.
11. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.
12. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Assay Specificity and Species Reactivity

The antibodies used within this ELISA kit detect mouse IL-6.
Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN-γ, IGFBP-3, IGFBP-5, IGFBP-6, IL-1a, IL-1β, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1a, MIP-1γ, MIP-2, MIP-3β, MIP-3a, PF-4, P-Selectin, RANTES, SCF, SDF-1a, TARC, TCA-3, TECK, TIMP-1, TNF- a,TNFRI,TNFRII,TPO,VCAM-1,VEGF).

Technical Support

For technical support contact information, visit: www.abcam.com/contactus