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ab100710
Mouse IL-4 ELISA Kit

For the quantitative measurement of mouse IL-4 in cell lysates and tissue lysates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Abcam's Mouse IL-4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse IL-4 in cell lysate and tissue lysates.

This assay employs an antibody specific for mouse IL-4 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL- 4 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse IL-4 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 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

⇩

Add standard or sample to appropriate wells. Incubate at room temperature.

⇩

Add prepared biotin antibody to each well. Incubate at room temperature.

⇩

Add prepared Streptavidin Solution. Incubate at room temperature.

⇩

Add TMB One-Step Development Solution to each well.

⇩

Add Stop Solution to each well. Read at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- 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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at -20°C immediately upon receipt.

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.

5. 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.

6. Materials Supplied

Item	Quantity	Storage Condition
IL-4 Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C
Recombinant mouse IL-4 Standard	2 Vials	-20°C
5X Sample Diluent Buffer	10 mL	-20°C
5X Assay Diluent	15 mL	-20°C
Biotinylated anti-mouse IL-4	2 Vials	-20°C
600X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C
2X Cell Lysis Buffer	5 mL	-20°C

7. Materials Required, Not Supplied

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

- 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.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Sample Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.

9. 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.

9.1 1X Assay Diluent

5X Assay Diluent should be diluted 5-fold with deionized or distilled water before use.

9.2 1X Sample Diluent Buffer

5X Sample Diluent Buffer should be diluted 5-fold with deionized or distilled water before use.

9.3 1X Cell Lysis Buffer

2X Cell Lysis Buffer is diluted to 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

9.4 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.5 1X Biotinylated IL-4 Detection Antibody

Briefly spin the Biotinylated anti-mouse IL-4 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 100-fold with 1X Assay Diluent prior to use in the Assay Procedure.

9.6 1X HRP-Streptavidin Solution

Briefly spin the 600X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP Streptavidin concentrate must be diluted 600-fold with 1X Assay Diluent prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 20 µL of 600X HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent to prepare a final 600 fold diluted 1X HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- Standard should be stored at -80°C after reconstitution.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Briefly spin the vial of IL-4 Standard. Prepare the 50 ng/mL Stock Standard by adding 400 µL 1X Sample Diluent Buffer into the vial (see table below).
10.2 Ensure the powder is thoroughly dissolved by gentle mixing.
10.3 Label tubes #1-8.
10.4 Prepare Standard #1 by adding 4 µL of the 50 ng/mL Stock Standard, to 996 µL of 1X Sample Diluent Buffer into tube 1#. Mix thoroughly and gently.
10.5 Pipette 450 µL 1X Sample Diluent Buffer into remaining tubes.
10.6 Prepare Standard #2 by adding 300 µL Standard #1 to tube #2 and mix thoroughly.
10.7 Prepare Standard #3 by adding 300 µL Standard #2 to tube #3 and mix thoroughly.
10.8 Using the table below as a guide, prepare further serial dilutions.
10.9 1X Sample Diluent Buffer serves as the zero standard (0 pg/mL).

Standard #	Volume to Dilute (µL)	Volume Diluent (µL)	Total Volume (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	4	996	1000	50000	200
2	300	450	750	200	80
3	300	450	750	80	32
4	300	450	750	32	12.8
5	300	450	750	12.8	5.12
6	300	450	750	5.12	2.05
7	300	450	750	2.05	0.819
8 (Blank)	-	450	450	-	-

11. Sample Preparation

General Sample Information:

- Tissue lysate and cell lysate sample should be diluted at least 5-fold with 1x Sample Diluent Buffer.

12. 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. Contents of each well can be recorded on the template sheet included in the Resources section.

13. 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.

13.1 Add 100 µL of each standard (see Standard Preparation, section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

13.2 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 auto washer. 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.

13.3 Add 100 µL of 1X Biotinylated IL-4 Detection Antibody (Reagent Preparation, section 9.4) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation section 9.5) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 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.

13.8 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. Calculation

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

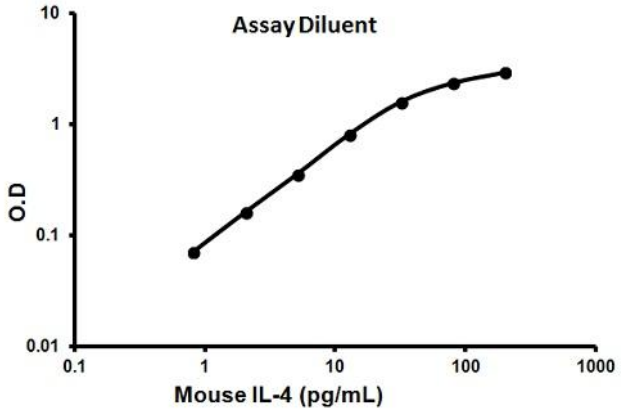

Figure 1. Example of a typical mouse IL-4 dilution series. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of Mouse IL-4 was determined to be 1 pg/ml.

PRECISION –

	Intra-assay Precision	Inter-Assay Precision
CV (%)	<10%	<12%

RECOVERY –

Recovery was determined by spiking various levels of mouse IL-4 into tissue lysate and cell lysate. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range %
Tissue Lysate	91.57	82-103
Cell Lysate	93.62	84-104

Linearity of Dilution –

Tissue Lysate Dilution	Average % Expected Value	Range (%)
1:2	92	82-105
1:4	94	83-104

Cell Lysate Dilution	Average % Expected Value	Range (%)
1:2	93	83-104
1:4	92	82-103

17. Assay Specificity

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, IGFBP-3, IGFBP-5, IGFBP-6, IL-1a, IL-1β, IL-2, IL-3, IL-3 Rb, IL-5, IL-6, 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).

18. Species Reactivity

This kit detects mouse IL-4 in cell and tissue lysate.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hours standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted proteins at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)