



## **Mouse IL-4 ELISA Kit**

Catalog No. EZMIL4

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures.

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## Introduction

Mouse IL-4 (Interleukin-4, Ia inducing factor (IaIF), B-cell stimulating factor-1,BSF-1, Hodgkin's cell growth factor, HCGF, Mast cell growth factor-2, MCGF-2, Macro- phage fusion factor, MFF, T cell growth factor-2, TCGF-2) is a pleiotropic cytokine that is produced by activated T cells, mast cells and basophils. IL-4 elicits many different biological responses, but has two dominant functions. IL-4 regulates differentiation of naïve CD4<sup>+</sup> T cell to Th2 cells. Th2 cells produce IL-4, IL-5, IL-10 and IL-13, which tend to favor a humoral immune response while suppressing a cell-mediated immune response controlled by Th1 cells. In addition, IL-4 regulates IgE and IgG1 production by B cells.

The EMD Millipore Mouse IL-4 ELISA Kit is a Sandwich Enzyme- Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a capture antibody. This kit is specifically designed for the accurate quantification of mouse IL-4 from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

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## Kit Components

- Mouse IL-4 Pre-Coated Plate (Part No. CS210071): One 96-well plate,
- Mouse IL-4 Detection Antibody (Part No. CS210035): One 12 mL bottle,
- Mouse IL-4 Standard (Part No. CS210036): One lyophilized vial,
- Matrix C (Part No. CS210060): One lyophilized vial
- Avidin HRP A (Part No. CS210051): One 12 mL bottle,
- Assay Buffer A (Part No. CS210062): One 25 mL bottle,
- Wash Buffer (20X) (Part No. CS210053): One 50 mL bottle,
- Substrate Solution F (Part No. CS210054): One 12 mL bottle,
- Stop Solution (Part No. CS213422): One 12 mL bottle,
- Plate Sealers (Part No. CS210056): One 4 pack.

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## Materials Not Supplied

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

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## Storage & Stability Information

Store kit and unopened components at 2°C to 8°C

Stability: Kit components are stable for a minimum of 4 months from the date of receipt if stored and handled as described below.

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## Reagent Precautions

- **Safety Warnings and Precautions:** This kit is designed for research use only and is not recommended for internal use in mice or animals. All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.
- This kit is not for use in diagnostic procedures.
- **Caustic Material: Stop Solution.** The stop solution contains acid which is harmful if swallowed or inhaled; avoid contact with skin and eyes; wash areas of contact immediately with water. **Caution: Eye, hand, face, and clothing protection should be worn when handling this material.**
- Substrate Solution F is harmful if inhaled or ingested. Additionally, avoid skin, eye or clothing contact with the substrate reagents
- The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and a failure to produce accurate data.

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## Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantification.

### Cell Culture Supernatant

If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

### Serum

Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x g. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

### Plasma

Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

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## Reagent Preparation

Follow the directions below to prepare reagents and solutions necessary for performing the Mouse IL-4 ELISA Kit.

1. **20X Wash Buffer:** The Wash Buffer provided is supplied as a 20X concentrate. Verify that it does not contain precipitate prior to diluting it to 1X. If necessary, warm to room temperature (20°C to 25°C) prior to dilution. Dilute the concentrate to 1X strength using Milli-Q™ or deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water.

2. Mouse IL-4 Standard: Reconstitute the lyophilized Mouse IL-4 Standard by adding the volume of Assay Buffer A indicated on the vial label to make the 20 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15-40 minutes, then briefly vortex to mix completely.
3. If serum or plasma will be assayed, reconstitute the lyophilized Matrix C by dispensing 2 mL of Milli-Q™ or deionized water into the vial and allow to sit at room temperature for 15 minutes, then vortex completely.

## Assay Protocol

1. Prepare the reagents as described in the Reagent Preparation section.
2. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Identify the number of reaction wells needed and remove any unneeded strip wells (8 wells per strip) from the plate holder. To do so carefully turn the plate upside down and push the strips from the bottom out through the plate. Re-seal the extra strips in the resealable bag with desiccant and store in the refrigerator for future use.
4. Prepare 500  $\mu$ L of the 125 pg/mL top standard by diluting 3.1  $\mu$ L of the standard stock solution in 496.9  $\mu$ L of Assay Buffer A. Perform six two-fold serial dilutions of the 125 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the mouse IL-4 standard concentrations in the tubes are 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, 7.8 pg/mL, 3.9 pg/mL, and 2 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).

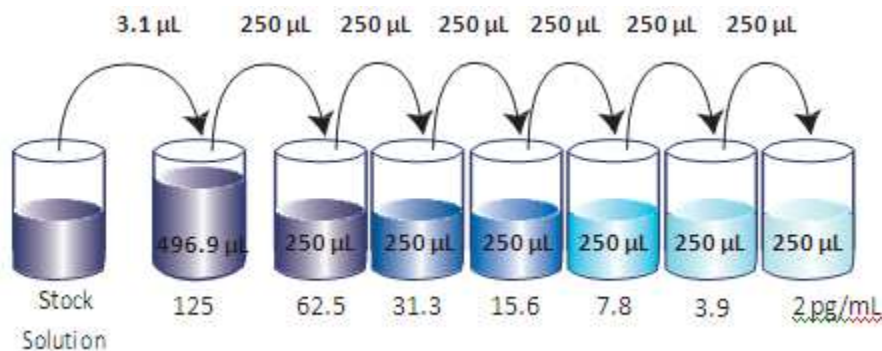


Figure 1: Schematic of serial dilutions and concentrations (pg/mL) for preparation of standards

5. Wash the plate 4 times with at least 300  $\mu$ L of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
6. For measuring samples of cell culture supernatant add 50  $\mu$ L of Assay Buffer A to each well that will contain either standard dilutions or samples.
7. For measuring serum or plasma samples, add 50  $\mu$ L of Matrix to each well that will contain the standards. Add 50  $\mu$ L of Assay Buffer A to each well that will contain the samples.
8. Add 50  $\mu$ L of standard dilutions or samples to the appropriate wells.
9. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100  $\mu$ L of Mouse IL-4 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

12. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
13. Add 100  $\mu$ L of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
14. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
15. Add 100  $\mu$ L of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing mouse IL-4 should turn blue in color with intensity proportional to its concentration. It is not necessary to seal the plate during this step.
16. Stop the reaction by adding 100  $\mu$ L of Stop Solution to each well. The solution color should change from blue to yellow.
17. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

# Assay Procedure Summary

## Assay Procedure Summary

1. Wash 4 times  
Add 50  $\mu$ L Assay Buffer A  

2. Add 50  $\mu$ L diluted standards or samples  
Incubate 2 hr, RT, shaking  

3. Wash 4 times  
Add 100  $\mu$ L Detection Antibody solution  
Incubate 1 hr, RT, shaking  

4. Wash 4 times  
Add 100  $\mu$ L Avidin-HRP A solution  
Incubate 30 min, RT, shaking  

5. Wash 5 times  
Add 100  $\mu$ L Substrate Solution  
Incubate 15 min, RT, in the dark  

6. Add 100  $\mu$ L Stop Solution  

7. Read absorbance at 450 nm and 570 nm

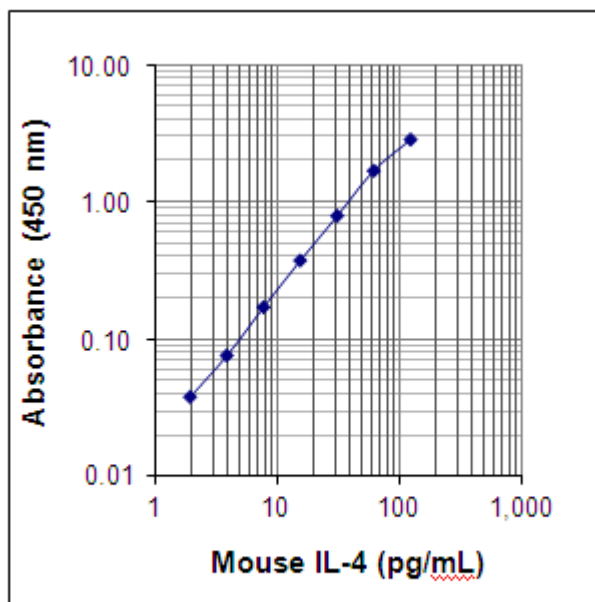
## Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

## Typical Data:

This standard curve was generated for demonstration purposes only. A standard curve must be run with each assay.



## Performance Characteristics:

### Specificity

No cross-reactivity was observed when this kit was used to analyze mouse recombinant cytokines/chemokines at up to 50 ng/mL.

### Sensitivity

The minimum detectable concentration of IL-4 is 0.5 pg/mL.

### Recovery

Recombinant IL-4 (62.5 and 15.6 pg/mL) was spiked into 4 mouse serum samples, then analyzed. On average, 91% of the cytokine was recovered from serum samples.

### Linearity

Four mouse serum samples with high concentrations of IL-4 were diluted 1:1, 1:2, 1:4, 1:8 with Matrix C to produce samples with values within the dynamic range and then assayed. On average, 105 % of the expected cytokine was detected from serum samples.

### Intra-Assay Statistics

Sixteen replicates each of two different IL-4 concentrations were tested in one assay.

| Concentration              | Sample 1 | Sample 2 |
|----------------------------|----------|----------|
| Number of Replicates       | 16       | 16       |
| Mean Concentration (pg/mL) | 30.4     | 7.3      |
| Standard Deviation         | 1.4      | 0.4      |
| % CV                       | 4.6      | 5.3      |

### Inter-Assay Statistics

Sixteen replicates each of two different concentrations of IL-4 were tested on four different plates.

| Concentration              | Sample 1 | Sample 2 |
|----------------------------|----------|----------|
| Number of Assays           | 4        | 4        |
| Mean Concentration (pg/mL) | 31.2     | 6.9      |
| Standard Deviation         | 1.8      | 0.6      |
| % CV                       | 5.7      | 8.6      |

### Biological Samples

Mouse splenocytes ( $1 \times 10^6$  cells/mL) were stimulated with 10 mg/mL plate-coated CD3 plus 1 µg/mL soluble CD28 at 37°C for 2 days. Cell culture supernatants were collected and analyzed for the levels of natural mouse IL-4. Mouse IL-4 concentration is 1,050 pg/mL in CD3/CD28- stimulated samples and 3.8 pg/mL in unstimulated samples.



## Troubleshooting Guide

| Problem                                   | Probable Cause                                                                                                                                                                                           | Solution                                                                                                                                                                                                                   |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Background is high                        | Background wells were contaminated                                                                                                                                                                       | Avoid cross-well contamination by using the sealer appropriately.<br><br>Use multichannel pipettes without touching the reagents on the plate.                                                                             |
|                                           | Matrix used has endogenous analyte                                                                                                                                                                       | Check the matrix ingredients for cross reacting components (e.g. interleukin modified tissue culture medium).                                                                                                              |
|                                           | Insufficient washes                                                                                                                                                                                      | Increase number of washes.<br><br>Increase soaking time between washes prior to addition of substrate solution                                                                                                             |
|                                           | TMB Substrate Solution was contaminated                                                                                                                                                                  | TMB Substrate Solution should be clear and colorless prior to addition to wells.<br><br>Use a clean container prior to pipetting substrate solution into wells.                                                            |
| No Signal                                 | Incorrect or no Detection Antibody was added<br><br>Avidin-HRP was not added.<br>Substrate solution was not added<br><br>Wash buffer contains sodium azide                                               | Add appropriate Detection Antibody and continue<br><br>Add Avidin-HRP according to protocol and continue<br><br>Add substrate solution and continue<br><br>Avoid sodium azide in the Wash Buffer.                          |
| Low or poor signal for the standard curve | Standard was incompletely reconstituted or was inappropriately stored<br>Reagents added to wells with incorrect concentrations<br><br>Incubations done at inappropriate temperature, timing or agitation | Reconstitute standard according to protocol. Store reconstituted standard in appropriate vials and store at -70°C<br><br>Check for pipetting errors and correct reagent volume.<br><br>Assay conditions need to be checked |

| Problem                                               | Probable Cause                                                            | Solution                                                                                                                                                                                                                    |
|-------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Signal is high, standard curves have saturated O.D.'s | Standard reconstituted with less volume than required                     | Reconstitute lyophilized standard with correct volume of solution recommended in the protocol.                                                                                                                              |
|                                                       | Plate incubation was too long                                             | Decrease incubation time                                                                                                                                                                                                    |
|                                                       | Detection antibody incubation time is too long                            | Decrease detection antibody incubation time                                                                                                                                                                                 |
|                                                       | Avidin-HRP incubation time is too long                                    | Decrease Avidin HRP incubation time                                                                                                                                                                                         |
|                                                       | Substrate solution incubation time is too long                            | Decrease Avidin HRP incubation time                                                                                                                                                                                         |
| Sample readings are out of range                      | Sample contain no or below detectable levels of analyte                   | If samples are below detectable levels it may be possible to use higher sample volume.                                                                                                                                      |
|                                                       | Sample contain analyte concentrations greater than highest standard point | Sample may require dilution and reanalysis                                                                                                                                                                                  |
| High variation in sample and/or standards             | Multichannel pipette errors                                               | Calibrate the pipettes                                                                                                                                                                                                      |
|                                                       | Plate washing was not adequate or uniform                                 | Make sure pipette tips are tightly secured. Confirm all reagents are removed completely in all wash steps.                                                                                                                  |
|                                                       | Non-homogenous samples                                                    | Thoroughly mix samples before pipetting.                                                                                                                                                                                    |
|                                                       | Samples may have high particulate matter                                  | Remove the particulate matter by centrifugation.                                                                                                                                                                            |
|                                                       | Insufficient plate agitation                                              | The plate should be agitated during all incubation steps using an ELISA plate shaker at a speed where solutions in wells are within constant motion without splashing.                                                      |
|                                                       | Cross-well contamination                                                  | When reusing plate sealers check that no reagent has touched the sealer. Care should be taken when using the same pipette tips used for reagent additions. Ensure that pipette tips do not touch the reagents on the plate. |

Microtiter Plate Map

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |
| G |   |   |   |   |   |   |   |   |   |    |    |    |
| H |   |   |   |   |   |   |   |   |   |    |    |    |

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