Mouse IL-4 ELISA Kit

For the quantitative determination of mouse interleukin-4 (IL-4) concentrations in mouse serum, cell culture supernatant, and other biological fluids

MEC1009-2 *(192 tests × 1)*
MEC1009-6 *(192 tests × 3)*

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (01/15-02) mIL-4 [2/6]

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INTENDED USE

This Mouse IL-4 ELISA kit is to be used for the *in vitro* quantitative determination of mouse interleukin 4 (IL-4) concentrations in serum, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY.

INTRODUCTION

IL-4 is a very important B cell stimulation and differentiation factor. In addition, IL-4 modulates the immune function of other cell types including T cells, monocytes, macrophages, mast cells, fibroblasts, endothelial cells, osteoblasts, keratinocytes, hepatocytes, and astrocytes. IL-4 is mainly produced by CD4+ TH0 and TH2 cells, CD8+ T cells, and mast cells. IL-4 is the polarizing cytokine for TH2 phenotype.

The gene for mouse IL-4 is mapped to chromosome 11 in close conjunction with genes for other T-helper 2 cytokines such as IL-13, GM-CSF, IL-5. Mouse IL-4 is a disulfide bond linked homodimer containing 113 amino acids in each subunit. The receptor for IL-4 is a heterodimer with a cytokine specific alpha unit, and a signal transduction beta subunit that is identical to the beta subunit of receptors for IL-13 and GM-CSF. IL-4 receptor alpha (IL-4Ra) is expressed by eosinophils, B cells and basophils in member-bound form and soluble form. While the membrane bound form participates in the activation of eosinophils and B cells, the soluble form of IL-4Ra has an antagonist effect to IL-4. Increased IL-4 expression causes chronic IgE response and hypereosinophilic syndromes. Elevated IL-4 level is found in allergic diseases such as allergic rhinitis, asthma and allergic conjunctivitis.

This ELISA kit provides a tool for studying IL-4 expression and regulation in animal model.

PRINCIPLE OF THE ASSAY

This mouse IL-4 enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for mouse IL-4. When standards or samples are added to the appropriate microtiter plate wells, mouse IL-4 in the standards or samples will be immobilized by the precoated antibody during incubation. Then, a biotin-conjugated antibody preparation specific for mouse IL-4 is added to each well and incubated. The biotin labelled antibody attaches to the wells by binding to mouse IL-4. After plate washing, other proteins, components and unattached biotin labelled antibody is removed. After that, avidin-horseradish peroxidase (HRP) conjugate is added to each well. Avidin has a very high affinity for biotin, thus, it links the tracer (HRP) sturdily to the biotin labelled antibody. The wells are thoroughly washed to remove all unbound avidin-HRP conjugate and a TMB (3,3', 5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation

period. Only wells that contain mouse IL-4 will exhibit a change in colour. The extent of colour change is proportional to the quantity of mouse IL-4 present in the standards/samples. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wave length of 450 nm ± 2 nm.

In order to measure the concentration of mouse IL-4 in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus IL-4 concentration (pg/mL). The concentration of mouse IL-4 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

This mouse IL-4 ELISA is a 3.5-hour solid-phase immunoassay readily applicable to measure mouse IL-4 levels in serum, cell culture supernatant, and other biological fluids in the range of 7.8 to 500pg/mL.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	192 tests × 1	***192 tests × 3***

1. **MOUSE IL-4 MICROTITER PLATE** (Part MEC09-1) ***96 wells × 2*** ***96 wells × 6***
   Pre-coated with anti-mouse IL-4 monoclonal antibody

2. **BIOTIN CONJUGATE** (Part MEC09-2) ***12 mL*** ***12 mL × 3***
   Anti-mouse IL-4 monoclonal antibody conjugated to biotin

3. **AVIDIN-HRP CONJUGATE** (Part MEC09-3) ***24 mL*** ***24 mL × 3***
   Avidin conjugated to horseradish peroxidase

4. **MOUSE IL-4 STANDARD** (Part MEC09-4) ***4 vials*** ***4 vials × 3***
   Recombinant mouse IL-4 (500pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part MEC09-5) ***50 mL*** ***50 mL × 3***
   Animal serum with preservative. *For serum testing*.

6. **CALIBRATOR DILUENT II** (Part MEC09-6) ***50 mL*** ***50 mL × 3***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (30X)** (Part 30009) ***60 mL*** ***60 mL × 3***
   30-fold concentrated solution of buffered surfactant.

8. **TMB SUBSTRATE** (Part 30010) ***20 mL*** ***20 mL × 3***
   Ready to use

9. **STOP SOLUTION** (Part 30008) ***28 mL*** ***28 mL × 3***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL required for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Mouse serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from mouse blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate mouse viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Crystal formation in the wash buffer (30X) will not affect its quality. Prior to dilution, make sure all crystals are completely dissolved by warming to 37°C.

SAMPLE PREPARATION

1. COLLECTION, HANDLING, AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

- Avoid hemolytic, lipidic or turbid samples.
- Samples must be stored at -20°C (short term) or -70°C (long term) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- Serum and cell culture supernatant are to be thawed immediately before use.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

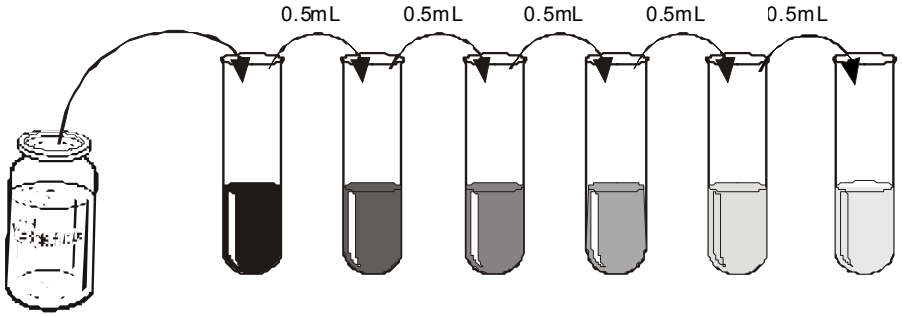
1. **Wash Buffer (1X):** Dissolving the crystals in Wash Buffer (30X) by warming up to 37°C. Add 60 mL of Wash Buffer (30X) and dilute to a final volume of 1800 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (30X) to 29 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Mouse IL-4 Standard:**

   a) Two vials of Standards are provided in this kit to allow both serum and cell culture supernatant testing. Reconstitute the mouse IL-4 Standard with either 1.0mL of Calibrator Diluent I (for serum testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 500pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. Aliquot the stock and store frozen at -20°C (short term) or -70°C (long term) if repeated use is expected.

   b) Use the above stock solution to produce a 2-fold serial dilution within the range of this assay (7.8pg/mL to 500pg/mL) as illustrated. Add 0.5mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to

mix contents thoroughly. The undiluted IL-4 standard will serve as the high standard (500pg/mL) and the Calibrator Diluent will serve as the zero-standard (0pg/mL).



500pg/mL 250pg/mL 125pg/mL 62.5pg/mL 31.25 pg/mL 15.6pg/mL 7.8pg/mL

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and mouse IL-4 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 - **0 pg/mL** (S1)	**2A, 2B**	Standard 5 – **62.5pg/mL** (S5)
1C, 1D	Standard 2 – **7.8pg/mL** (S2)	**2C, 2D**	Standard 6 – **125pg/mL** (S6)
1E, 1F	Standard 3 – **15.6pg/mL** (S3)	**2E, 2F**	Standard 7 - **250pg/mL** (S7)
1G, 1H	Standard 4 – **31.25pg/mL** (S4)	**2G,2H**	Standard 8 - **500pg/mL** (S8)
		3A-12H	Mouse IL-4 samples

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 50µL of Standard or sample to the appropriate well of anti-mouse IL-4 antibody pre-coated microtiter plate. Cover and incubate for 1 hour at room temperature.

3. Without discarding the standards and samples, add 50μL anti-mouse IL-4 biotin conjugate to each well. Mix well. Cover and incubate for 1 hour at room temperature.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100μL of Avidin-HRP conjugate to each well. Cover and incubate for 1 hour at room temperature.

6. Repeat wash procedure as described in Step 4.

7. Add 100μL TMB to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION RESULT

The standard curve is used to determine the amount of mouse IL-4 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-4 concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of IL-4 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-4 concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

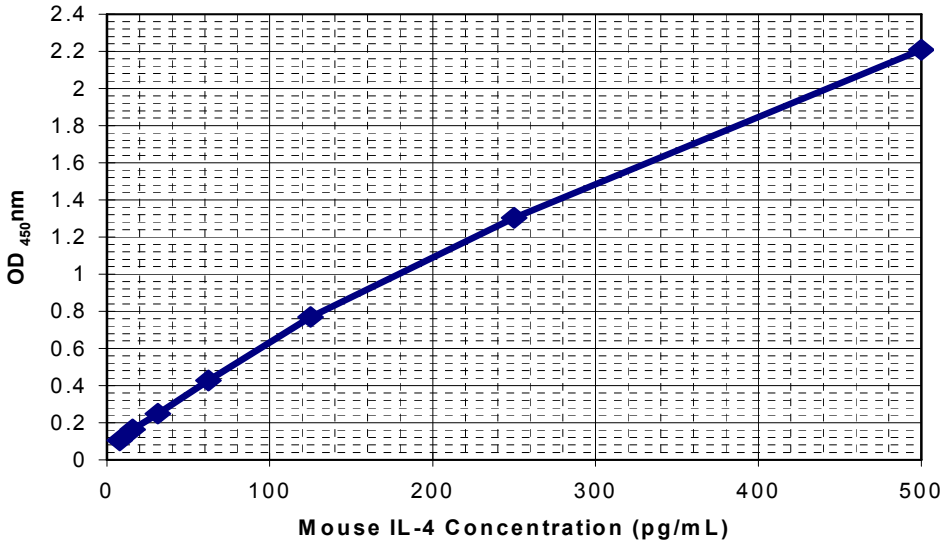
TYPICAL DATA

Results of a typical standard run of a mouse IL-4 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate user results.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

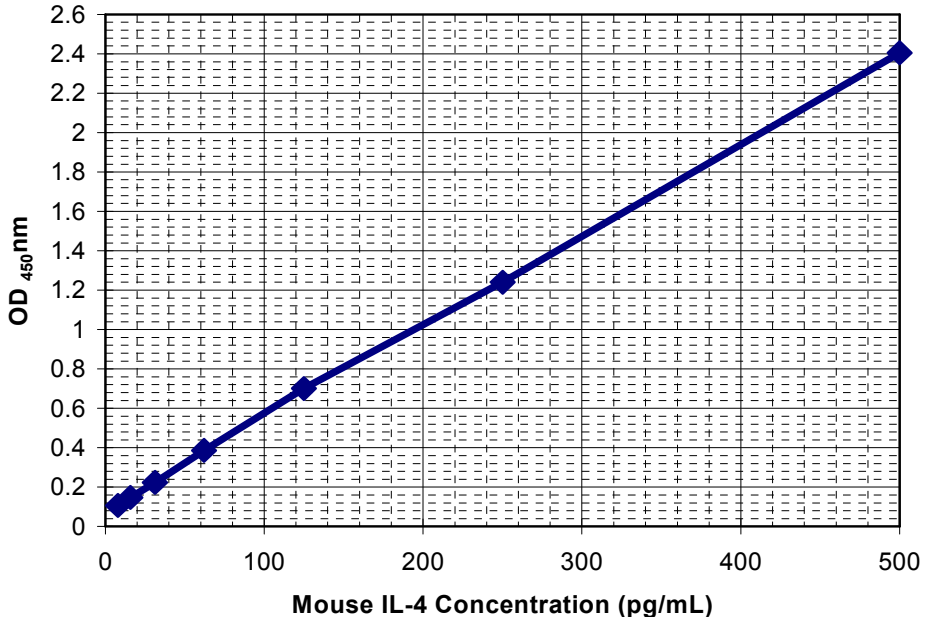
Standard (pg/mL)	**Mean O.D. (450 nm)**	**%CV**	**Zero Standard Subtracted** (Std.)-(S1)
0	0.0530	0	0
7.8	0.1045	3.38	0.0515
15.6	0.1625	1.31	0.1095
31.25	0.2485	1.99	0.1959
62.5	0.4265	1.49	0.3210
125	0.7690	2.76	0.7160
250	1.3025	5.59	1.2495
500	2.2075	2.08	2.1545



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.)-(S1)
0	0.0690	1.23	0
7.8	0.1070	6.61	0.038
15.6	0.1485	6.19	0.0795
31.25	0.2250	1.26	0.1560
62.5	0.3900	1.45	0.3210
125	0.7065	2.50	0.6375
250	1.2485	1.64	1.1795
500	2.4290	0.17	2.3600



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by using 16 replicates in 1 assay.

Sample	1	2	3
N	16	16	16
Mean (pg/ml)	263.334	69.237	18.350
Coefficient of Variation (%)	4.66	4.91	5.02

2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were assayed by using replicates on 8 different assays.

	Calibrator Diluent II assay		
Sample	**1**	**2**	**3**
N	8	8	8
Mean (pg/mL)	240.976	65.055	14.715
Coefficient of Variation (%)	5.22	4.65	7.15

3. RECOVERY

The recovery of mouse IL-4 within cell culture media and mouse serum was evaluated with mouse IL-4 spiked samples. The recovery of IL-4 in mouse serum was low (20%), which may be caused by the presence of soluble IL-4 receptor in serum.

Sample Type	Average Recovery %	Range %
Cell culture media	102.9	94 -113.5

4. SENSITIVITY

The minimum detectable dose of mouse IL-4 was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of mouse IL-4 calculated from calibrate I diluted standard curve was <0.8pg/mL.

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