

Mouse IL-4 ELISA Kit

EMIL4 EMIL42 EMIL45

1345.3

| Number | Description |
|---------------|--|
| EMIL4 | <p>Mouse Interleukin-4 (IL-4) ELISA Kit, sufficient reagents for 96 determinations</p> <p>Kit contents:</p> <p>Anti-Mouse IL-4 Pre-coated 96-well Strip Plate, 1 each</p> <p>Lyophilized Recombinant Mouse IL-4 Standard, 2 vials</p> <p>Standard Diluent, 12 ml, contains 0.1% sodium azide</p> <p>Plate Reagent, 8 ml, contains 0.1% sodium azide</p> <p>30X Wash Buffer, 50 ml</p> <p>Prediluted Conjugate Reagent, 12 ml</p> <p>TMB Substrate Solution, 13 ml</p> <p>Stop Solution, 13 ml, contains 0.16 M sulfuric acid</p> <p>Adhesive Plate Covers, 6 each</p> |
| EMIL42 | <p>Mouse Interleukin-4 (IL-4) ELISA Kit, sufficient reagents for 2 × 96 determinations</p> <p>Kit contents:</p> <p>Anti-Mouse IL-4 Pre-coated 96-well Strip Plate, 2 each</p> <p>Lyophilized Recombinant Mouse IL-4 Standard, 4 vials</p> <p>Standard Diluent, 2 × 12 ml, contains 0.1% sodium azide</p> <p>Plate Reagent, 2 × 8 ml, contains 0.1% sodium azide</p> <p>30X Wash Buffer, 2 × 50 ml</p> <p>Prediluted Conjugate Reagent, 2 × 12 ml</p> <p>TMB Substrate Solution, 2 × 13 ml</p> <p>Stop Solution, 2 × 13 ml, contains 0.16 M sulfuric acid</p> <p>Adhesive Plate Covers, 12 each</p> |
| EMIL45 | <p>Mouse Interleukin-4 (IL-4) ELISA Kit, sufficient reagents for 5 × 96 determinations</p> <p>Kit contents:</p> <p>Anti-Mouse IL-4 Pre-coated 96-well Strip Plate, 5 each</p> <p>Lyophilized Recombinant Mouse IL-4 Standard, 5 vials</p> <p>Standard Diluent, 55 ml, contains 0.1% sodium azide</p> <p>Plate Reagent, 35 ml, contains 0.1% sodium azide</p> <p>30X Wash Buffer, 200 ml</p> <p>Prediluted Conjugate Reagent, 55 ml</p> <p>TMB Substrate Solution, 5 × 13 ml</p> <p>Stop Solution, 55 ml, contains 0.16 M sulfuric acid</p> <p>Adhesive Plate Covers, 30 each</p> |

For research use only. Not for use in diagnostic procedures.

Storage: For maximum stability, store in a non-defrosting -20°C freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8°C and refer to the expiration date for refrigerated storage. Once thawed, store at 4°C until the expiration date for refrigerated storage. Kit is shipped on dry ice.



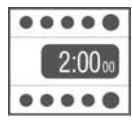



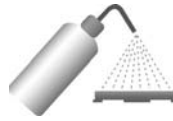




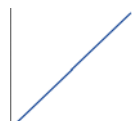
Introduction

The Thermo Scientific Mouse Interleukin-4 (IL-4) ELISA is an *in vitro* enzyme-linked immunosorbent assay for quantification of mouse IL-4 in serum and culture supernatants.

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Procedure Summary

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| 1. Add 50 µl of Plate Reagent to each well. | 2. Add 50 µl of Standards or samples to each well in duplicate. | 3. Cover plate and incubate at 37°C for 2 hours in humidified incubator. | 4. Wash plate FIVE times. |
|  |  |  |  |
| 5. Add 100 µl of Prediluted Conjugate Reagent to each well. | 6. Cover plate and incubate at 37°C for 1 hour in humidified incubator. | 7. Wash plate FIVE times. | 8. Add 100 µl Premixed TMB Substrate Solution to each well. |
|  |  |  |  |
| 9. Develop plate in the dark at room temperature (20-25°C) for 30 minutes. | 10. Stop reaction by adding 100 µl of Stop Solution to each well. | 11. Measure absorbance. Subtract 550 nm values from 450 nm values. | 12. Calculate results using graph paper or curve-fitting statistical software. |

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1,000 µl and plastic pipettes to deliver 5-15 ml
- A glass or plastic 2 liter container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5 ml polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- A standard ELISA reader for measuring absorbance at 450 nm and 550 nm. If a 550 nm filter is not available, the absorbance can be measured at 450 nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

Precautions

- All samples and reagents must be equilibrated to room temperature (20-25°C) before use in the assay.
- Review these instructions carefully and verify all components against the Kit Contents list (page 1) before beginning.
- Do not use a water bath to thaw samples. Thaw samples at room temperature.
- Use a humidified 37°C incubator for sample and conjugate incubations.
- When preparing standard curve and sample dilution in culture medium, use the same medium used to culture the cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to dilute the standard and samples. Do NOT use RPMI without serum supplement.
- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- To avoid cross-contamination, use new disposable pipette tips for each transfer and a new adhesive plate cover for each incubation step.
- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Discard unused ELISA components after assay completion. Do not mix reagents from different kit lots.
- Do not use glass pipettes to measure TMB Substrate Solution. Take care not to contaminate the Solution. If it is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some kit components contain sodium azide. Please dispose of reagents according to local regulations.

Additional Precautions for the 2-plate and 5-plate Kits

- Dispense only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.
- Use only one bottle of the TMB Substrate Solution per 96-well plate. Do not combine leftover substrate with that reserved for other plates.
- Equilibrate to room temperature only the reagent volumes required for the number of plates being used.
- For the 5-plate kit, use only one vial of Standard per 96-well plate. The 2-plate kit is supplied with four vials of Standard. Therefore, four partial-plate assays may be performed.

Sample Preparation

Sample Handling

- Serum and culture supernatants may be tested in this ELISA. Use 50 µl per well of serum or culture supernatant.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C.
- Avoid repeated freeze-thaw cycles when storing samples.
- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use a heated water bath to thaw or warm samples.
- Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.

Sample Dilution

- If the IL-4 concentration possibly exceeds the highest point of the standard curve (i.e., 375 pg/ml), prepare one or more five-fold dilutions of the sample. When testing **culture supernatants**, prepare the dilutions using the culture medium. When testing **serum**, prepare the serial dilution using the Standard Diluent provided. For example, prepare a five-fold dilution by adding 100 µl of sample to 400 µl of appropriate diluent. Mix thoroughly between dilutions before assaying.

Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout this instruction booklet.

Wash Buffer

1. Label a clean glass or plastic 2 liter container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.
2. If using a 5-plate kit, add 30 ml Wash Buffer to 870 ml of water for each plate being used, otherwise, add the entire contents of the 30X Wash Buffer bottle and dilute to a final volume of 1.5 liters with ultrapure water. Mix thoroughly. Wash Buffer must be equilibrated to room temperature before use in the assay.

(PP) When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

Note: Wash buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

Standards

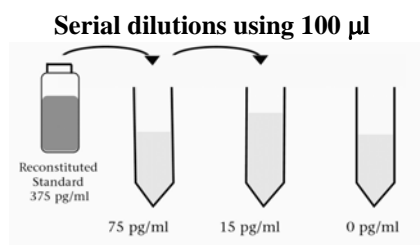
- **(PP)** Reconstitute and use one vial of the lyophilized Standard per partial plate.
- Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.

When testing **culture supernatant samples**, reconstitute standard in sample culture medium. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the sample culture medium to prepare Standard Curve dilutions. The concentration will be 375 pg/ml, which is the highest point of the standard curve.

When testing **serum samples**, reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. The concentration will be 375 pg/ml, which is the highest point of the standard curve. Use the Standard Diluent provided to prepare standard curve serial dilutions.

When testing both **serum and cell culture supernatant samples on the same plate**, validate the media to establish if the same standard curve may be used for both sample types. Prepare a standard curve (including a zero/blank) using culture medium to reconstitute and dilute the standard. Perform this curve in parallel with a standard curve reconstituted in ultrapure water and diluted in the Standard Diluent provided. If the OD values of the two curves are within 10% of the mean for both curves, then the assay may be performed using either curve.

1. Label three tubes, one for each of the remaining standard curve points: 375 pg/ml, 75 pg/ml, 15 pg/ml and 0 pg/ml, then prepare 1:5 serial dilutions for the standard curve as follows:
2. Pipette 400 µl of appropriate diluent into each tube.
3. Pipette 100 µl of the reconstituted standard into the first tube (i.e., 75 pg/ml) and mix.
4. Pipette 100 µl of this dilution into the second tube (i.e., 15 pg/ml) and mix.



Assay Procedure

A. Sample Incubation

- **(PP)** Determine number of strips required. Leave these strips in the plate frame. Tightly seal unused strips in the provided foil pouch with desiccant and store at 2-8°C. After completing assay, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.
- Use the Data Template provided to record the locations of the zero standard (blank or negative control), IL-4 standards and test samples. Perform three standard points and one blank in duplicate with each series of unknown samples.

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- If using a multichannel pipettor, use a new reagent reservoir to add the Plate Reagent. Remove from the vial only the amount required for the number of strips being used.
1. Add 50 μ l of Plate Reagent to all wells being used.
 2. Add 50 μ l of reconstituted standards or test samples in duplicate to each well.
Note: If the mouse IL-4 concentration in any test sample possibly exceeds the highest point on the standard curve, 375 pg/ml, see Sample Preparation – Sample Dilution section.
 3. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over edges and down each strip. Incubate for two (2) hours at 37°C in a humidified incubator.
 4. Carefully remove adhesive plate cover. Wash plate FIVE times with Wash Buffer as described in the Plate Washing section (section B).

B. Plate Washing

1. Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.
2. Empty plate contents. Use a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure four additional times for a total of FIVE washes. Blot plate onto paper towels or other absorbent material.
Note: For automated washing, aspirate all wells and wash FIVE times with Wash Buffer, over-filling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

C. Conjugate Incubation

1. Add 100 μ l of Prediluted Conjugate Reagent to each well. If using a multichannel pipettor, use a new reagent reservoir when adding the Conjugate Reagent.
2. Carefully attach a new adhesive plate cover. Make sure all edges and strips are tightly sealed. Incubate plate for one (1) hour at 37°C in a humidified incubator.
3. Carefully remove adhesive plate cover and wash FIVE times as described in the Plate Washing section.

D. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding the TMB Substrate Solution and Stop Solution.
 - **(PP)** Do not combine leftover substrate with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate Solution
1. Dispense from bottle ONLY amount required, 100 μ l per well, for the number of wells being used. Do not use a glass pipette to measure the TMB Substrate Solution.
 2. Pipette 100 μ l of TMB Substrate Solution into each well.
 3. Allow color reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
 4. After 30 minutes, stop the reaction by adding 100 μ l of Stop Solution to each well.

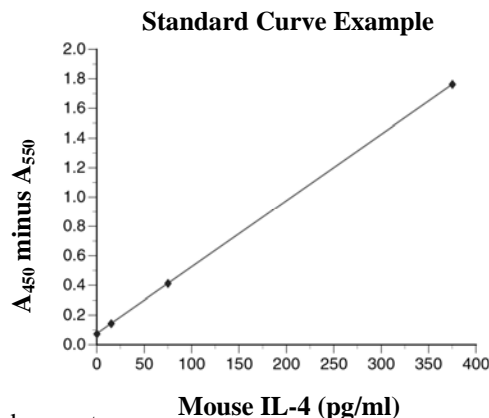
E. Absorbance Measurement

Note: Evaluate the plate within 30 minutes of stopping the reaction.

Measure absorbance on an ELISA plate reader set at 450 nm and 550 nm. Subtract 550 nm values from 450 nm values to correct for optical imperfections in the microplate. If absorbance at 550 nm is unavailable, measure the absorbance at 450 nm only. When the 550 nm measurement is omitted, absorbance values will be higher.

F. Results Calculations

- Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding IL-4 concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the IL-4 amount in each sample by interpolating from the absorbance value (Y axis) to IL-4 concentration (X axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate pg/ml of IL-4 in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.



Performance Characteristics

Sensitivity: < 5 pg/ml

The sensitivity or Lower Limit of Detection (LLD)¹ was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Assay Range: 15-375 pg/ml

Suggested standard curve points are 0, 15, 75 and 375 pg/ml.

Reproducibility:

Intra-assay CV: < 10%

Inter-assay CV: < 10%

Specificity: Natural and recombinant mouse IL-4. This ELISA does not cross-react with mouse IL-2, IL-3, IL-5, IL-6, IL-7, IFN γ , GM-CSF, TNF α ; rat IL-4; rabbit IL-4; or human IL-4.

Calibration: The standard in this ELISA assay is calibrated to NCI BRB reference standard 91/656.

One (1) pg = one (1) NCI BRB pg.

Reference

1. In *Immunoassay: A Practical Guide*, Chan and Perlstein, Eds., 1987, Academic Press: New York, p71.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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Data Templates

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

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
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