

Mouse IL-5 ELISA Kit

EMIL5 EMIL52 EMIL55

1364.4

Number	Description
EMIL5	Mouse Interleukin-5 (IL-5) ELISA Kit , sufficient reagents for 96 determinations Kit Contents: Anti-Mouse IL-5 Pre-coated 96-well Strip Plate , 1 each Lyophilized Recombinant Mouse IL-5 Standard , 2 vials Standard Diluent , 12 ml, contains 0.1% sodium azide Plate Reagent , 8 ml, contains 0.1% sodium azide 30X Wash Buffer , 50 ml Anti-Mouse IL-5 Peroxidase Conjugate Reagent , 12 ml TMB Substrate , 13 ml Stop Solution , 13 ml, contains 0.16 M sulfuric acid Adhesive Plate Covers , 6 each
EMIL52	Mouse Interleukin-5 (IL-5) ELISA Kit , sufficient reagents for 2 × 96 determinations Kit Contents: Anti-Mouse IL-5 Pre-coated 96-well Strip Plate , 2 each Lyophilized Recombinant Mouse IL-5 Standard , 4 vials Standard Diluent , 2 × 12 ml, contains 0.1% sodium azide Plate Reagent , 2 × 8 ml, contains 0.1% sodium azide 30X Wash Buffer , 2 × 50 ml Anti-Mouse IL-5 Peroxidase Conjugate Reagent , 2 × 12 ml TMB Substrate , 2 × 13 ml Stop Solution , 2 × 13 ml, contains 0.16 M sulfuric acid Adhesive Plate Covers , 12 each
EMIL55	Mouse Interleukin-5 (IL-5) ELISA Kit , sufficient reagents for 5 × 96 determinations Kit Contents: Anti-Mouse IL-5 Precoated 96-well Plate Strip , 5 each Lyophilized Recombinant Mouse IL-5 Standard , 5 vials Standard Diluent , 55 ml, contains 0.1% sodium azide Plate Reagent , 35 ml, contains 0.1% sodium azide 30X Wash Buffer , 200 ml Anti-Mouse IL-5 Peroxidase Conjugate Reagent , 55 ml TMB Substrate , 5 × 13 ml Stop Solution , 55 ml, contains 0.16 M sulfuric acid Adhesive Plate Covers , 30 each

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.




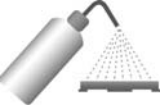

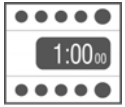
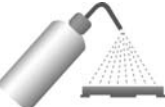





Introduction

This Thermo Scientific ELISA is for measuring mouse IL-5 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 Peroxidase 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 of the TMB Substrate to each well. |
| 
9. Develop plate in the dark at room temperature (20-25°C) for 30 minutes. | 
10. Add 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 humidified incubator
- A glass or plastic two-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 at room temperature (20-25°C) before use in the assay.
- Review all instructions carefully and verify components against the Kit Contents list (page 1) before beginning assay.
- Do not use water a bath to thaw samples. Thaw samples at room temperature.
- 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.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use 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.
- For sample and conjugate incubations use a humidified 37°C incubator.
- Avoid microbial contamination of reagents.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Discard unused kit components. 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 the solution 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 2-plate and 5-plate Kits

- Dispense only 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 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 or culture supernatants may be tested in this ELISA.
- 50 µl per well of serum or culture supernatant are required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C.
- Avoid repeat 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 mouse IL-5 concentration possibly exceeds the highest point of the standard curve (i.e., 320 pg/ml), prepare one or more five-fold dilutions of the test sample. When testing **culture supernatants**, prepare the serial dilutions using the culture medium. When testing **serum**, prepare the serial dilutions using the Standard Diluent provided. For example, prepare a five-fold dilution is adding 100 μ l of sample to 400 μ l of appropriate diluent. Mix thoroughly between dilutions before assaying.

Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout the instructions.

Note: When using the 5-plate kit, only one standard per plate is supplied, therefore, partial plates cannot be used.

Wash Buffer

1. Label a clean glass or plastic two-liter container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.
2. If using the 5-plate kit, add 30 ml Wash Buffer to 870 ml water for each plate used, otherwise add the entire contents of the 30X Wash Buffer (50 ml) bottle to the two-liter container and dilute to a final volume of 1.5 liters with ultrapure water. Mix thoroughly.

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

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

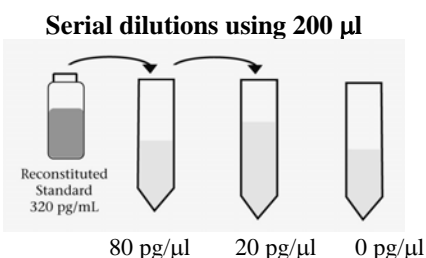
Standards

- **(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 with the cell culture medium. Reconstitution volume is stated on the vial label. The standard dissolves in approximately 1 minute and will be 320 pg/ml, which is the highest point of the standard curve. Mix by gently inverting vial. Use the sample culture medium to prepare dilutions.

When testing **serum samples**, reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard dissolves in approximately 1 minute and will be 320 pg/ml, which is the highest point of the standard curve. Mix by gently inverting vial. Use the Standard Diluent provided to prepare the serial dilutions.

When testing **both serum and cell culture supernatant samples on the same plate**, validate the medium 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. Use medium containing serum or other protein to maximize IL-5 stability. 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 with Standard Diluent, whether testing culture supernatant or serum samples.

1. Label three tubes, one for each of the remaining standard curve points: 80 pg/ml, 20 pg/ml, and 0 pg/ml, then prepare 1:4 serial dilutions for the standard curve as follows:
2. Pipette 600 μ l of appropriate diluent into each tube.
3. Pipette 200 μ l of the reconstituted standard into the first tube (i.e., 80 pg/ml) and mix.
4. Pipette 200 μ l of this dilution into the second tube (i.e., 20 pg/ml) and mix.



Assay Procedure

A. Sample Incubation

- **(PP)** Determine number of strips required. Leave these strips in the plate frame. Tightly seal remaining 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 locations of the zero standard (blank or negative control), mouse IL-5 standards and test samples. Perform three standard points and one blank in duplicate with each series of samples.
- 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. Take care not to touch the samples in wells with the pipette tip when adding the Plate Reagent.

1. Add 50 µl of Plate Reagent to each well being used.
2. Add 50 µl of reconstituted standards or test samples in duplicate to each well. Mix well by gently tapping the plate several times.

Note: If the mouse IL-5 concentration in any test sample exceeds the highest point on the standard curve, 320 pg/ml, see Sample Preparation – Sample Dilution section.

3. Add 50 µl of Standard Diluent to all wells that do not contain standards or samples.
4. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are sealed tightly by running your thumb over edges and down each strip. Incubate for two (2) hours at 37°C in a humidified incubator.
5. Carefully remove adhesive plate cover. Wash plate as described in the Plate Washing section below.

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, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

C. Peroxidase Conjugate Reagent Incubation

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

D. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate and Stop Solution.
 - Dispense from bottle **ONLY** amount required, 100 µl per well, for the number of wells being used. Do not use a glass pipette to measure the TMB Substrate.
 - **(PP)** Do not combine leftover substrate with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate.
1. Add 100 µl of TMB Substrate into each well.
 2. 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.
 3. After 30 minutes, stop the reaction by adding 100 µl of Stop Solution to each well.

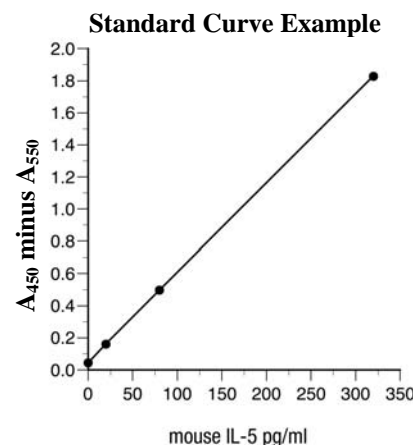
E. Absorbance Measurement

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

Measure the 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 Calculation

- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding IL-5 concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the mouse IL-5 amount in each sample by interpolating from the absorbance value (Y axis) to IL-5 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 mouse IL-5 in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean. Carefully consider duplicate values that differ from the mean by greater than 10%.



Performance Characteristics

Sensitivity: < 5 pg/ml

Assay Range: 20-320 pg/ml

Suggested standard curve points are 320, 80, 20, and 0 pg/ml.

Reproducibility:

Intra-assay CV: < 10%

Inter-assay CV: < 10%

Specificity: This ELISA is specific for the measurement of natural and recombinant mouse IL-5. This ELISA does not cross-react with mouse IL-2, IL-3, IL-4, IL-6, IFN γ , GM-CSF, TNF α , rat IL-5, rabbit IL-5 or human IL-5.

Serum Recovery: Recovery is determined by spiking recombinant cytokine into undiluted pooled mouse serum and comparing it with a spiked PBS/BSA control. Typical recoveries are as follows:

Control Values	Serum Values	Recovery
201 pg/ml	163 pg/ml	81%
85 pg/ml	67 pg/ml	79%
41 pg/ml	36 pg/ml	88%
20 pg/ml	17 pg/ml	86%

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There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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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												
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