

Mouse IL-1 β ELISA Kit

EMIL1B EMIL1B2 EMIL1B5

1363.3

Number	Description
EMIL1B	<p>Mouse Interleukin-1 beta (IL-1β) ELISA Kit, sufficient reagents for 96 determinations</p> <p>Kit Contents:</p> <p>Anti-Mouse IL-1β Precoated 96-well Strip Plate, 1 each</p> <p>Lyophilized Recombinant Mouse IL-1β Standard, 2 vials</p> <p>Standard Diluent, 12 ml</p> <p>Biotinylated Antibody Reagent, 8 ml</p> <p>30X Wash Buffer, 50 ml</p> <p>Streptavidin-HRP Concentrate, 100 μl</p> <p>Streptavidin-HRP Dilution Buffer, 14 ml</p> <p>TMB Substrate, 13 ml</p> <p>Stop Solution, 13 ml, contains 0.16 M sulfuric acid</p> <p>Adhesive Plate Covers, 6 each</p>
EMIL1B2	<p>Mouse IL-1β ELISA Kit, sufficient reagents for 2 \times 96 determinations</p> <p>Kit Contents:</p> <p>Anti-Mouse IL-1β Precoated 96-well Strip Plate, 2 each</p> <p>Lyophilized Recombinant Mouse IL-1β Standard, 4 vials</p> <p>Standard Diluent, 2 \times 12 ml</p> <p>Biotinylated Antibody Reagent, 2 \times 8 ml</p> <p>30X Wash Buffer, 2 \times 50 ml</p> <p>Streptavidin-HRP Concentrate, 2 \times 100 μl</p> <p>Streptavidin-HRP Dilution Buffer, 2 \times 14 ml</p> <p>TMB Substrate, 2 \times 13 ml</p> <p>Stop Solution, 2 \times 13 ml, contains 0.16 M sulfuric acid</p> <p>Adhesive Plate Covers, 12 each</p>
EMIL1B5	<p>Mouse IL-1β ELISA Kit, sufficient reagents for 5 \times 96 determinations</p> <p>Kit Contents:</p> <p>Anti-Mouse IL-1β Precoated 96-well Strip Plate, 5 each</p> <p>Lyophilized Recombinant Mouse IL-1β Standard, 5 vials</p> <p>Standard Diluent, 55 ml</p> <p>Biotinylated Antibody Reagent, 30 ml</p> <p>30X Wash Buffer, 200 ml</p> <p>Streptavidin-HRP Concentrate, 250 μl</p> <p>Streptavidin-HRP Dilution Buffer, 70 ml</p> <p>TMB Substrate, 5 \times 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.



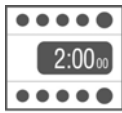








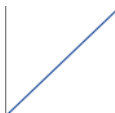
Introduction

This Thermo Scientific ELISA Kit is for measuring mouse IL-1 β in culture supernatants, EDTA plasma, sodium citrate plasma, heparin plasma and serum.

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Procedure Summary

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| 1. Add 50 μ l of Biotinylated Antibody Reagent to each well. | 2. Add 50 μ l of standards or samples to each well in duplicate. | 3. Cover plate and incubate at room temperature (20-25°C) for 2 hours. | 4. Wash plate THREE times. |
|  |  |  |  |
| 5. Add 100 μ l of prepared Streptavidin-HRP Concentrate to each well. | 6. Cover plate and incubate at room temperature for 30 minutes. | 7. Wash plate THREE times. | 8. Add 100 μ l of the TMB Substrate to each well. |
|  |  |  |  |
| 9. Develop plate in the dark at room temperature for 30 minutes. | 10. Stop reaction by adding 100 μ l of Stop Solution to each well. | 11. Measure absorbance on a plate reader at 450 nm minus 550 nm. | 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 μ l and plastic pipettes to deliver 5-15 ml
- A glass or plastic two-liter container to prepare Wash Buffer
- A squirt wash bottle or an automated plate washer
- 1.5 ml polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- 15 ml plastic tube and a microcentrifuge to prepare the Streptavidin-HRP Solution
- 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°) before use in the ELISA.**
- Review all instructions carefully and verify components against the Kit Contents list (page 1) before beginning the assay.
- Do not use a water 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 a new adhesive plate cover for each incubation step to avoid cross-contamination.
- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.
- 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. 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.

Additional Precautions for the 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.
- Note:** The 2-plate kit is supplied with four vials of standard. Therefore, four partial plate assays may be performed.

Sample Preparation

Sample Handling

- Serum; EDTA, heparin and sodium citrate plasma; or culture supernatants may be tested in this ELISA.
- 50 µl per well of serum, plasma 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 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 heated water baths 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-1 β concentration possibly exceeds the highest point of the standard curve (i.e., 1,000 pg/ml), prepare one or more 10-fold dilutions of the test sample. When testing **culture supernatants** prepare the serial dilutions using the culture medium. When testing **serum or plasma**, prepare the serial dilutions using the Standard Diluent provided. For example, a 10-fold dilution is prepared by adding 50 µl of test sample to 450 µl of appropriate diluent. Mix samples thoroughly between dilutions before assaying.

Reagent Preparation

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

Note: When using 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 a 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 the solution thoroughly.

(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.

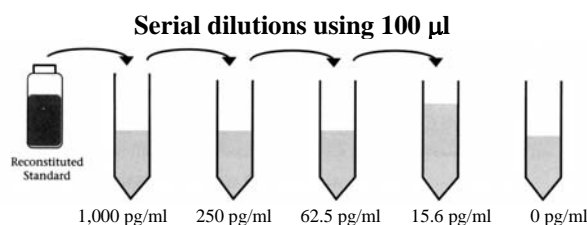
Standards

- **(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.
1. When testing **culture supernatant 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. Use the sample culture medium to prepare standard curve serial dilutions.

When testing **serum or plasma 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. Use the Standard Diluent provided to prepare standard curve serial dilutions.

When testing **serum or plasma and cell culture supernatant samples on the same plate**, validate the media to establish if the same standard curve can be used for the different 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 mouse IL-1 β 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, plasma or serum.

2. Label 5 tubes, one for each standard curve point: 1,000 pg/ml, 250 pg/ml, 62.5 pg/ml, 15.6 pg/ml, and 0 pg/ml, then prepare 1:4 serial dilutions for the standard curve as follows:
3. Pipette 300 μ l of appropriate diluent into each tube.
4. Pipette 100 μ l of the reconstituted standard into the first tube (i.e., 1,000 pg/ml) and mix.
5. Pipette 100 μ l of this dilution into the second tube (250 pg/ml) and mix.
6. Repeat the serial dilutions (using 100 μ l) two more times to complete the standard curve points.



Assay Procedure

A. Biotinylated Antibody Reagent and 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 the locations of the zero standard (blank or negative control), mouse IL-1 β standards and test samples. Perform four 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 Biotinylated Antibody 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 Biotinylated Antibody Reagent.
1. Add 50 μ l of Biotinylated Antibody Reagent to each well.
 2. Add 50 μ l 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-1 β concentration in any test sample exceeds the highest point on the standard curve, 1,000 pg/ml, see Sample Preparation–Sample Dilution section.

3. Add 50 μ l 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 room temperature, 20-25°C.
5. Carefully remove adhesive plate cover. Wash plate **THREE** 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 two additional times for a total of **THREE** washes. Blot plate onto paper towels or other absorbent material.

Note: For automated washing, aspirate all wells and wash **THREE** times with Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

C. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution **immediately before use**. Do not prepare more solution than required. Do not store prepared Streptavidin-HRP Solution.
 - Use a 15 ml plastic tube to prepare Streptavidin-HRP Solution.
 - If using a multichannel pipettor, **use new reagent reservoir and pipette tips** when adding the prepared Streptavidin-HRP Solution.
1. Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom.
 2. **(PP)** Use only the Streptavidin-HRP Solution amount required for the number of strips being used. For each strip, mix 2.5 μ l of Streptavidin-HRP Concentrate with 1 ml of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

For one complete 96-well plate, add 30 μ l of Streptavidin-HRP Concentrate to 12 ml of Streptavidin-HRP Dilution Buffer and mix gently.

3. Add 100 μ l of prepared Streptavidin-HRP Solution to each well.
4. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
5. Carefully remove the adhesive plate cover, discard plate contents and wash **THREE** times as described in the Plate Washing section (section B).

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. Pipette 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.

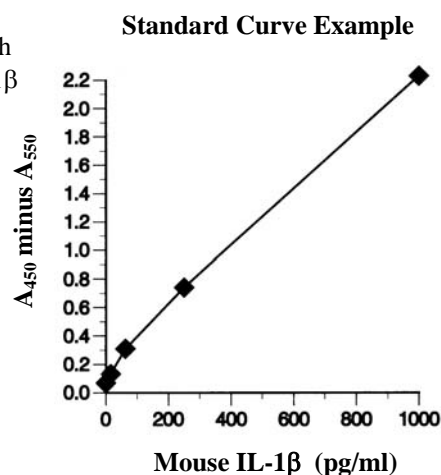
E. Absorbance Measurement

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 an absorbance at 550 nm is not available, measure the absorbance at 450 nm only. When the 550 nm measurement is omitted, absorbance values will be higher.

F. Calculation of Results

- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding mouse IL-1β concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the mouse IL-1β amount in each sample by interpolating from the absorbance value (Y axis) to mouse IL-1β 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 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: < 3 pg/ml

The sensitivity or lower limit of detection (LLD)¹ is 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.

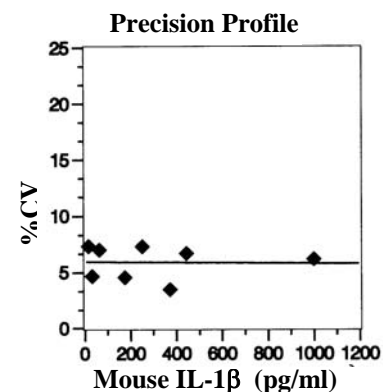
Assay Range: 15.6-1,000 pg/ml

Suggested standard curve points are 1,000, 250, 62.5, 15.6, 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-1β. This ELISA does not cross-react with mouse IL-1α, IL-3, IL-4, IL-5, IL-7, IL-10, TNFα, IFNγ, GM-CSF or human IL-1β.

Calibration: The standard in this ELISA is calibrated to NIBSC standard lot 93/668.
One (1) pg of internal standard = 0.8 NIBSC pg = 0.8 NIBSC units.

Precision: The intra-assay coefficient of variation is plotted against mouse IL-1 β concentration (pg/ml). The points represent samples evaluated in replicates of four in four different kit lots.

Expected Values: The mean level of mouse IL-1 β in 31 serum samples collected from apparently healthy mice was 12 pg/ml, ranging from 0 to 82 pg/ml. The mean level of mouse IL-1 β in 12 plasma samples collected from apparently healthy mice was 1.6 pg/ml, ranging from 0 to 16 pg/ml.

Reference

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

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Data Templates

	1	2	3	4	5	6	7	8	9	10	11	12
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C												
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