INSTRUCTIONS

Mouse IL-1β ELISA Kit

EM2IL1B EM2IL1B2 EM2IL1B5

1363.6

Number	Description
EM2IL1B	**Mouse Interleukin-1 beta (IL-1β) ELISA Kit,** sufficient reagents for 96 determinations
EM2IL1B2	**Mouse IL-1β ELISA Kit,** sufficient reagents for 2 × 96 determinations
EM2IL1B5	**Mouse IL-1β ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	EM2IL1B	EM2IL1B2	EM2IL1B5
Anti-mouse IL-1β Precoated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Mouse IL-1β Standard	2 vials	4 vials	5 vials
Standard Diluent	12mL	2 × 12mL	55mL
Biotinylated Antibody Reagent	12mL	2 × 12mL	55mL
20X Wash Buffer	55mL	2 × 55mL	250mL
Streptavidin-HRP Concentrate	75µL	2 × 75µL	250µL
Streptavidin-HRP Dilution Buffer	13mL	2 × 13mL	70mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 2N sulfuric acid	6mL	2 × 6mL	30mL
Adhesive Plate Covers	6 each	12 each	30 each

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

Storage: For maximum stability, store in a non-defrosting -20ºC freezer and refer to the expiration date for frozen storage on the label. Kit is shipped on dry ice.

Table of Contents

Introduction

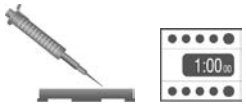
This Thermo Scientific ELISA Kit is for measuring mouse IL-1β in culture supernatants, EDTA plasma, sodium citrate plasma, heparin plasma and serum.

Procedure Summary

1. Add 100µL of standards or samples to each well in duplicate.

2. Cover plate and incubate at room temperature (20-25°C) for 2 hours.

3. Wash plate THREE times.



4. Add 100µL of Biotinylated Antibody Reagent to each well. Cover plate and incubate at room temperature for 1 hour.

5. Wash plate THREE times.

6. Add 100µL of prepared Streptavidin-HRP Solution to each well.

7. Cover plate and incubate at room temperature for 30 minutes.

8. Wash plate THREE times.

9. Add 100µL of TMB Substrate to each well.

10. Develop plate in the dark at room temperature for 30 minutes.

11. Add 50µL of Stop Solution to each well.

12. Measure absorbance on a plate reader at 450nm minus 550nm. Calculate results using curve-fitting statistical software.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL and plastic pipettes to deliver 5-15mL
- A glass or plastic two-liter container to prepare Wash Buffer
- A squirt wash bottle or an automated plate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- 15mL plastic tube and a microcentrifuge to prepare the Streptavidin-HRP Solution
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance can be measured at 450nm only. Refer to the instruction manual supplied with the instrument being used.
- Computerized curve-fitting statistical software package

Precautions

- **IMPORTANT: All samples and reagents must be at room temperature (20-25°) before use in the ELISA. Once thawed, mix reagents gently by inversion to ensure homogeneity.**
- 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.
- 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 exposing reagents to microbial contamination and 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.
- Coated plate wells may appear clear to hazy. Haziness does not impact product performance.

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. The 2-plate kit is supplied with four vials of standard. Therefore, four partial plate assays may be performed.

Sample Preparation

- Serum; EDTA, heparin and sodium citrate plasma; or culture supernatants may be tested in this ELISA.
- **Dilute samples two-fold in Standard Diluent before assaying.** For example, a two-fold dilution is prepared by adding 50µL of test sample to 50µL of Standard Diluent. Mix thoroughly before assaying.
- If the mouse IL-1β concentration possibly exceeds the highest point of the standard curve (i.e., 2000pg/mL), prepare one or more five-fold dilutions of the test sample using the Standard Diluent provided. For example, a five-fold dilution is prepared by adding 50µL of test sample to 200µL of Sample Diluent. Mix thoroughly between dilutions before assaying.
- 100µL per well of diluted serum, plasma or culture supernatant is 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.

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 20X Wash Buffer may have a cloudy appearance.
2. If using a 5-plate kit, add 40mL Wash Buffer to 760mL water for each plate used, otherwise, add 50mL of the 20X Wash Buffer bottle to the two-liter container and dilute to a final volume of 1.0L 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.

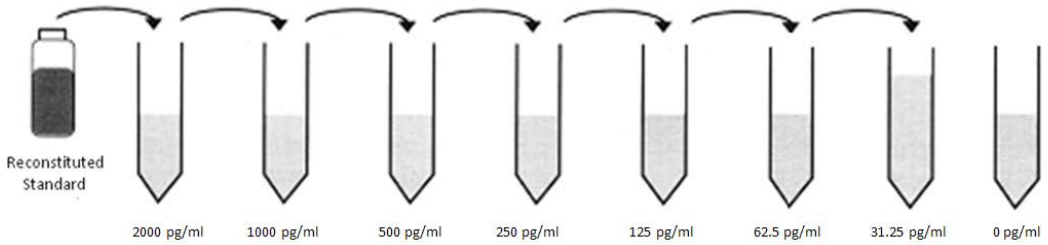
Standards

- **(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.

  Reconstitute the 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 reconstituted standard will be at 4000pg/mL.** Use the Standard Diluent provided to prepare serial dilutions for the standard curve. Note: It is recommended to prepare the standard curve in Standard Diluent for all sample types; however, if you are preparing a standard curve without using the Standard Diluent, perform the curve in parallel with a curve reconstituted in the Standard Diluent. If the OD values are within 10% of the mean of both curves, the standards may be prepared without using the Standard Diluent.

1. Label eight tubes, one for each standard curve point: 2000, 1000, 500, 250, 125, 62.5, 31.25 and 0pg/mL. Prepare 1:2 serial dilutions for the standard curve as follows:
2. Pipette 250µL of Standard Diluent into each tube.
3. Pipette 250µL of the reconstituted standard into the first tube (i.e., 2000pg/mL) and mix.
4. Pipette 250µL of this dilution into the second tube (1000pg/mL) and mix.
5. Repeat the serial dilutions (using 250µL) five more times to complete the standard curve points.

Serial dilutions using 250µL



Assay Procedure

A. Sample Incubation

- **(PP)** Determine the 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-1β standards and test samples. Perform seven standard points and one blank in duplicate with each series of unknowns.

1. Add 100µL of reconstituted standards or test samples (diluted two-fold in Standard Diluent, see Sample Preparation Section) in duplicate to each well. Mix well by gently tapping the plate several times.

   Note: If the mouse IL-1β concentration in any sample possibly exceeds the highest point on the standard curve, 2000pg/mL, see the Sample Preparation Section.
2. Carefully cover plate with a new adhesive plate cover. Ensure all edges and strips are sealed tightly by running your thumb over edges and down each strip. Incubate for 2 hours at room temperature, 20-25°C.
3. 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. Biotinylated Antibody Reagent Incubation

- If using a multichannel pipettor, **use a new reagent reservoir and pipette tips** when adding 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 100µL of Biotinylated Antibody Reagent to each well containing sample or standard.
2. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 1 hour at room temperature, 20-25°C.
3. Carefully remove the adhesive plate cover, discard plate contents and wash THREE times as described in the Plate Washing Section (Section B).

D. 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 15mL 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 2.5µL of Streptavidin-HRP Concentrate with 1mL of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

   For one complete 96-well plate, add 30µL of Streptavidin-HRP Concentrate to 12mL of Streptavidin-HRP Dilution Buffer and mix gently.
3. Add 100µL 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).

E. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate and Stop Solution.
- Dispense from bottle ONLY amount required, 100µL 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.

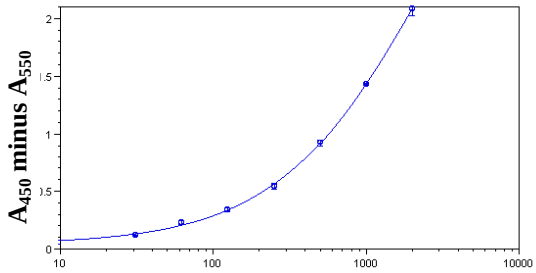
1. Pipette 100µL of TMB Substrate into each well.
2. Allow color reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
3. After 30 minutes, stop the reaction by adding 50µL of Stop Solution to each well.

F. Absorbance Measurement

Note: Evaluate the plate within 15 minutes of stopping the reaction.

Measure the absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If an absorbance at 550nm is not available, measure the absorbance at 450nm only. When the 550nm measurement is omitted, absorbance values will be higher.

Standard Curve Example



Mouse IL-1β (pg/mL)

G. 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 curve-fitting statistical software (four-parameter curve shown). 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: < 3pg/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.

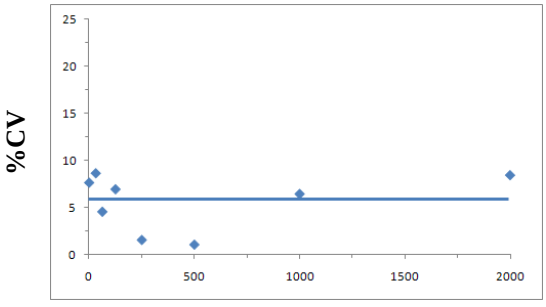
Assay Range: 31.25-2000pg/mL
Suggested standard curve points are 2000, 1000, 500, 250, 125, 62.5, 15.6 and 0pg/mL.

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 (≥ 1%) with mouse IL-1α, IL-10, IL-2, IL-4, IL-6, TNFα, GM-CSF; human IL-1β, IL-8, IL-1α; or bovine IL-1β. This ELISA does cross-react with rat IL-1β (12%).

Precision: The intra-assay coefficient of variation is plotted against mouse IL-1β concentration (pg/mL). The points represent samples evaluated in triplicate from a representative kit lot.

Precision Profile



Mouse IL-1β (pg/mL)

Cited 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
A												
B												
C												
D												
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F												
G												
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