

Mouse IL-2 ELISA Kit

EMIL2 EMIL22 EMIL25

1343.3

Number	Description
EMIL2	Mouse Interleukin-2 (IL-2) ELISA Kit , sufficient reagents for 96 determinations Kit Contents: Anti-Mouse IL-2 96-well Strip Plate , 1 each Lyophilized Recombinant Mouse IL-2 Standard , 2 vials Standard Diluent , 12 ml, contains 0.1% sodium azide Plate Reagent , 12 ml, contains 0.1% sodium azide 30X Wash Buffer , 50 ml Anti-IL2-HRP Conjugate Concentrate , 0.10 ml Conjugate Dilution Buffer , 14 ml TMB Substrate , 13 ml Stop Solution , 13 ml, contains 0.16 M sulfuric acid Adhesive Plate Covers , 6 each
EMIL22	Mouse Interleukin-2 ELISA Kit , sufficient reagents for 2 × 96 determinations Kit Contents: Anti-Mouse IL-2 96-well Strip Plate , 2 each Lyophilized Recombinant Mouse IL-2 Standard , 4 vials Standard Diluent , 2 × 12 ml, contains 0.1% sodium azide Plate Reagent , 2 × 12 ml, contains 0.1% sodium azide 30X Wash Buffer , 2 × 50 ml Anti-IL2-HRP Conjugate Concentrate , 2 × 0.1 ml Conjugate Dilution Buffer , 2 × 14 ml TMB Substrate , 2 × 13 ml Stop Solution , 2 × 13 ml, contains 0.16 M sulfuric acid Adhesive Plate Covers , 12 each
EMIL25	Mouse Interleukin-2 ELISA Kit , sufficient reagents for 5 × 96 determinations Kit Contents: Anti-Mouse IL-2 96-well Strip Plate , 5 each Lyophilized Recombinant Mouse IL-2 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-IL2-HRP Conjugate Concentrate , 0.4 ml Conjugate Dilution Buffer , 65 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.




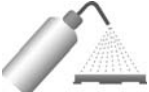


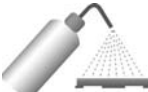




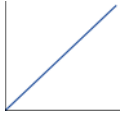
Introduction

The Thermo Scientific Mouse Interleukin-2 (IL-2) ELISA is an *in vitro* enzyme-linked immunosorbent assay for quantification of mouse IL-2 in serum and culture supernatants.

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Procedure Summary

 Step 1. Add 50 µl of Plate Reagent to each well.	 Step 2. Add 50 µl of Standards or Samples to each well in duplicate.	 Step 3. Cover plate and incubate at 37°C for 2 hours in a humidified incubator.	 Step 4. Wash plate FIVE times.
 Step 5. Add 100 µl of prepared Conjugate Reagent to each well.	 Step 6. Cover plate and incubate at 37°C for 1 hour in humidified incubator.	 Step 7. Wash plate FIVE times.	 Step 8. Add 100 µl TMB Substrate to each well.
 Step 9. Develop plate in the dark at room temperature (20-25°C) for 30 minutes.	 Step 10. Add 100 µl of Stop Solution to each well.	 Step 11. Measure absorbance on a plate reader at 450 minus 550 nm.	 Step 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 µl
- A glass or plastic 2 liter container to prepare Wash Buffer
- A squirt wash bottle or an automated immunoplate washer
- 1.5 ml polypropylene or polyethylene tubes to prepare standards - do not use polystyrene, polycarbonate or glass tubes
- 15 ml plastic tube and a microcentrifuge to prepare the Conjugate Reagent
- 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 read at 450 nm only. Refer to the instruction manual supplied with your instrument.
- 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.
- Do not use water a bath to thaw samples. Thaw samples at room temperature.
- Review these instructions carefully and verify all components against the Kit Contents list (page 1) before beginning.
- 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 and 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.
- 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.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Do not use glass pipettes to measure TMB Substrate Solution. Care must be taken not to contaminate the TMB Substrate Solution. If the solution is blue before use, DO NOT USE IT.
- 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 and, therefore, four partial plate assays may be performed.

Sample Preparation

- Serum or culture supernatants may be tested in this ELISA.
- 50 µl of sample per well 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.
- Samples and standards should be assayed in duplicate each time the ELISA is performed.
- Avoid repeated freeze-thaw cycles when storing samples.
- Gradually equilibrate samples to room temperature before beginning assay.
- If testing samples that are clotted, grossly hemolyzed 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.
- If the IL-2 concentration possibly exceeds the highest point of the standard curve (i.e., 850 pg/ml), prepare one or more five-fold dilutions of the sample. When testing **culture supernatants**, prepare serial dilutions using the culture medium. When testing **serum**, prepare serial dilutions 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 running partial plates, look for **(PP)** throughout this instruction booklet.

Wash Buffer

1. Label a clean glass or plastic 2 liter container "Wash Buffer."
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) If using a partial plate, store the reconstituted Wash Buffer at 2-8°C. Do not use Wash Buffer if it becomes visibly contaminated during storage.

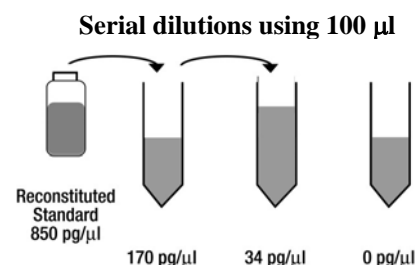
Standards

- **(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 in sample culture medium. Reconstitution volume is stated on the vial label. The standard dissolves in approximately 1 minute. Mix by gently inverting the vial. Use the sample culture medium to prepare Standard Curve dilutions. The concentration will be 850 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 vial label. The standard dissolves in approximately 1 minute. Mix by gently inverting vial. The concentration will be 850 pg/ml, which is the highest point of the standard curve. Use the Standard Diluent provided to prepare the serial dilutions for the standard curve.

When testing both **serum and cell culture supernatant samples on the same plate**, for best results, validate the media to establish if the same standard curve can 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.

2. Label three tubes, one for each of the remaining standard curve points: 170, 34 and 0 pg/ml, then prepare 1:5 serial dilutions for the standard curve as follows:
3. Add 400 µl of appropriate diluent into each tube.
4. Add 100 µl of the reconstituted standard into the first tube (i.e., 170 pg/ml) and mix.
5. Add 100 µl of this dilution into the second tube (i.e., 34 pg/ml) and mix.



Assay Procedure

A. Sample Incubation

- **(PP)** Determine number of strips required and leave these strips in the plate frame. Tightly seal the 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), IL-2 standards and test samples. Perform three standard points and one blank in duplicate with each series of unknown 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.
1. Add 50 µl of Plate Reagent to all wells being used.
 2. Add 50 µl of reconstituted standards or test samples in duplicate to each well.

Note: If the mouse IL-2 concentration in any test sample possibly exceeds the highest point on the standard curve (i.e., 850 pg/ml), see Sample Preparation Section.

- 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.
- Carefully remove adhesive plate cover. Wash plate FIVE times with Wash Buffer as described in the Plate Washing section below.

B. Plate Washing

- Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame.
- Manual Wash:** 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.
- Automated Wash:** 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 Reagent Preparation and Incubation

- Prepare Conjugate Reagent immediately before use. Do not prepare more Conjugate Reagent than required.
 - Do not store prepared Conjugate Reagent.
 - Use a 15 ml plastic tube to prepare Conjugate Reagent.
- Centrifuge Conjugate Concentrate to force entire vial contents to the bottom.
 - (PP)** Use only the Conjugate Reagent amount required for the number of strips being used. For each strip, mix 6 µl of Conjugate Concentrate with 1 ml of Conjugate Dilution Buffer. Store Conjugate Concentrate reserved for additional strips at 2-8°C.
 - For one complete 96-well plate, add 66 µl of Conjugate Concentrate to 11 ml of Conjugate Dilution Buffer and mix.
 - Use a single or multichannel pipettor to add 100 µl of prepared Conjugate Reagent to each well. If using a multichannel pipettor, use a new reagent reservoir when adding the Conjugate Reagent.
 - Carefully attach a new adhesive plate cover. Make sure all edges and strips are sealed tightly. Incubate plate for one (1) hour at 37°C in a humidified incubator.
 - 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 and Stop Solution.
 - Dispense from bottle **ONLY** amount required (i.e., 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.
- Pipette 100 µl of TMB Substrate into each well.
 - Allow enzymatic 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.
 - After 30 minutes, stop the reaction by adding 100 µl of Stop Solution to each well.

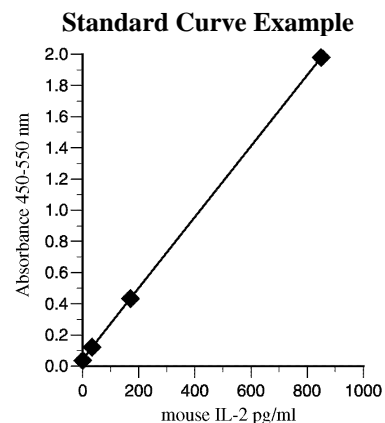
E. Absorbance Measurement

Note: The plate must be evaluated 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 at 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 IL-2 concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Use the standard curve to determine IL-2 amount in each sample by interpolating the absorbance value on the Y axis to IL-2 concentration on the X axis.
- 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-2 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 of this assay 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: 34-850 pg/ml

Suggested standard curve points are 0, 34, 170 and 850 pg/ml

Calibration: The standard in this ELISA is calibrated to NIH/NIBSC standard lot 93/566.

One (1) standard pg = 0.25 NIBSC pg = 0.025 NIBSC units.

Reproducibility:

Intra-assay CV: < 10%

Inter-assay CV: < 10%

Specificity: Natural and recombinant mouse IL-2. This ELISA does not cross-react with mouse IL-3, IL-4, IL-5, IL-6, IL-10, IFN γ , GM-CSF, TNF α or human IL-2.

References

1. In *Immunoassay: A Practical Guide*, Chan and Perlstein, Eds. 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												
E												
F												
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