INSTRUCTIONS

Mouse IL-10 ELISA Kit

EM2IL10 EM2IL102 EM2IL105

1346.6

Number	Description
EM2IL10	**Mouse Interleukin-10 (IL-10) ELISA Kit,** sufficient reagents for 96 determinations
EM2IL102	**Mouse Interleukin-10 (IL-10) ELISA Kit,** sufficient reagents for 2 × 96 determinations
EM2IL105	**Mouse Interleukin-10 (IL-10) ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	EM2IL10	EM2IL102	EM2IL105
Anti-Mouse IL-10 96-Well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Mouse IL-10 Standard	2 vials	4 vials	10 vials
Assay Buffer	12mL	2 × 12mL	5 × 12mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
Biotinylated Antibody Reagent	8mL	2 × 8mL	5 × 8mL
Streptavidin-HRP Concentrate	75µL	2 × 75µL	5 × 75µL
Streptavidin-HRP Dilution Buffer	14mL	2 × 14mL	5 × 14mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
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. Alternatively, store at 2-8ºC and refer to the expiration date for refrigerated storage. Once thawed, store at 4ºC until the expiration date for refrigerated storage. Kit is shipped on dry ice.

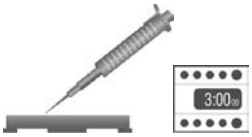
Table of Contents

Introduction

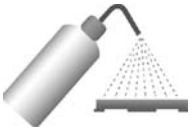
The Thermo Scientific™ Mouse Interleukin-10 (IL-10) ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of mouse IL-10 in serum and culture supernatants.

Procedure Summary

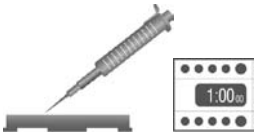
Step 1. Add 50µL of Assay Buffer to each well.



Step 2. Add 50µL of Standards or samples to each well in duplicate. Cover plate and incubate at room temperature (20-25°C) for 3 hours.



Step 3. Wash plate THREE times.



Step 4. Add 50µL of Premixed Biotinylated Antibody Reagent to each well. Cover plate and incubate at room temperature for 1 hour.

Step 5. Wash plate THREE times.

Step 6. Dilute Streptavidin-HRP Concentrate in Dilution Buffer. Add 100µL of this solution to each well.

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

Step 8. Wash plate THREE times.

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

Step 10. Develop plate at room temperature for 30 minutes.

Step 11. Stop reaction by adding 100µL of Stop Solution to each well.

Step 12. Measure the absorbance on a plate reader set at 450 and 550nm and calculate results.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-l000µL
- 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 to prepare Streptavidin-HRP Solution
- A microcentrifuge to prepare 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.
- 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 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.
- When assaying culture medium, prepare the standard curve and sample dilutions using the same medium used to culture cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to

dilute standard and samples. For best results, use a culture medium that contains a carrier protein such as FCS. Lack of a carrier protein in the media or addition of other compounds may compromise assay results.

- 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.
- Avoid microbial contamination of reagents.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Do not mix reagents from different kit lots. Discard unused components after assay completion.
- 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 Precaution for the 2-plate and 5-plate Kit

- Dispense, pool, and equilibrate to room temperature only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.

Sample Preparation

- Serum and culture supernatants may be tested in this assay.
- 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.
- If the mouse IL-10 concentration possibly exceeds the highest point of the standard curve (i.e., 3000pg/mL), prepare one or more 10-fold dilutions of the test sample. When testing **culture supernatants**, prepare the serial dilutions using culture medium. When testing **serum**, prepare the serial dilution using the Assay Buffer provided. For example, a 10-fold dilution is prepared by adding 50µL of test sample to 450µL of appropriate diluent. Mix thoroughly between dilutions before assaying.

Reagent Preparation

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

Wash Buffer

- **(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.

1. Label a clean glass or plastic two-liter container “Wash Buffer.” The 30X Wash Buffer may have a cloudy appearance.

2. Add the entire contents of the 30X Wash Buffer (50mL) bottle to the 2L container and dilute to a final volume of 1.5L with ultrapure water. Mix thoroughly.

Standards

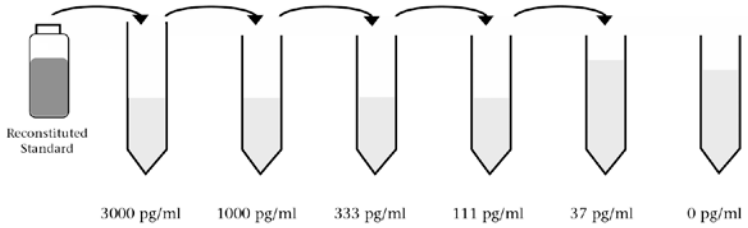
- **(PP)** Reconstitute and use one vial of the lyophilized Standard per partial plate.
- Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.
- When testing **culture supernatant samples**, reconstitute standard with ultrapure water. Reconstitution 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 dilutions.

  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. Use the Assay Buffer 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 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 prepared with Assay Buffer. If the OD values of the two curves are within 10% of the mean for both curves, then the assay can be performed with Assay Buffer, whether testing culture supernatant or serum samples.

1. Label six tubes, one for each standard curve point: 3000pg/mL, 1000pg/mL, 333pg/mL, 111pg/mL, 37pg/mL and 0pg/mL, then prepare 1:3 serial dilutions for the standard curve as follows:
2. Pipette 400µL of appropriate diluent into each tube.
3. Pipette 200µL of the reconstituted standard into the first tube (i.e., 3000pg/mL) and mix.
4. Pipette 200µL of this dilution into the second tube (i.e., 1000pg/mL) and mix.
5. Repeat serial dilutions four more times.

Standard Dilutions using 200µL



Assay Procedure

A. Sample Incubation

- **(PP)** Determine number of strips required. 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 the locations of the zero standard (blank or negative control), IL-10 standards and test samples. Perform five 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 Assay Buffer. Remove from the vial only the amount required for the number of strips being used.

1. Add 50µL of Assay Buffer to all wells being used.
2. Add 50µL of reconstituted standards or test samples in duplicate to each well.
3. **Note:** If the mouse IL-10 concentration in any test sample possibly exceeds the highest point on the standard curve, 3000pg/mL, dilute the sample as indicated in the Sample Preparation Section.
4. 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 3 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 the 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 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

Note: If using a multichannel pipettor, use a new reagent reservoir to add the Biotinylated Antibody Reagent.

(PP) Remove from the vial only the amount required for the number of strips being used.

1. Add 50µL of the Biotinylated Antibody Reagent to each well.
2. Carefully cover the plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over the edges and down each strip. Incubate for one (1) hour at room temperature, 20-25°C.
3. Carefully remove the adhesive plate cover. Wash the plate THREE times with Wash Buffer 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. Centrifuge Streptavidin-HRP Concentrate to force entire vial contents to the bottom.
2. For one complete 96-well plate, add 30µL of Streptavidin-HRP Concentrate to 12mL of Streptavidin-HRP Dilution Buffer and mix gently.
3. (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.
4. Add 100µL of prepared Streptavidin-HRP Solution to each well.
5. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate the plate for 30 minutes at room temperature, 20-25°C.
6. 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 the 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 Solution into each well.
2. Allow color 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 100µL of Stop Solution to each well.

F. Absorbance Measurement

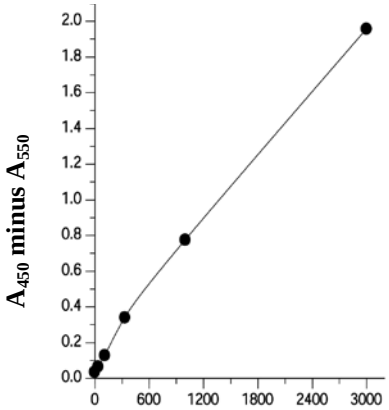
Note: Evaluate the plate within 30 minutes of stopping the reaction.

Measure 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.

G. Calculation of Results

- The standard curve is used to determine IL-10 amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding IL-10 concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the IL-10 amount in each sample by interpolating from the absorbance value (Y-axis) to 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-10 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%.

Example Standard Curve



Performance Characteristics

Sensitivity: < 12pg/mL of mouse IL-10
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: 37-3000pg/mL
Suggested standard curve points are 3000, 1000, 333, 111, 37 and 0pg/mL.

Reproducibility:
Intra-assay CV: < 10%
Inter-assay CV: < 10%

Specificity
The following cytokines do not interfere with or cross-react in the mouse IL-10 ELISA: mouse IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF, IFNγ, TNFα or human IL-10.

Recovery
Recovery in this ELISA was determined by spiking three different levels of recombinant mouse IL-10 into eight serum samples collected from apparently healthy Balb/c and NSA mice. Recoveries are as follows:

Spike Level	Mean Recovery
100pg/mL	116%
500pg/mL	102%
1500pg/mL	101%

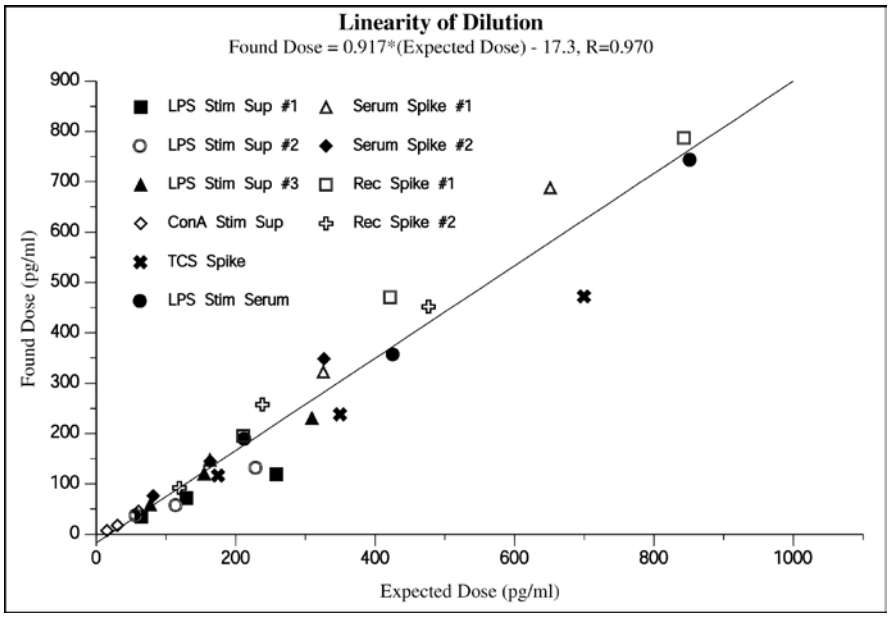
Expected Values*
The average level of mouse IL-10 detected in eight serum samples from apparently healthy Balb/c mice was 18pg/mL ranging from 0 to 53pg/mL. To induce mouse IL-10 production Balb/c mice were injected with LPS, 50µg/mouse. Serum samples were harvested by heart puncture during a three-hour time course and were then evaluated with the Mouse IL-10 ELISA. Additionally, splenocytes harvested from Balb/c mice were stimulated with 20µg/mL LPS during a three-day time course. Supernatants were collected at various time points and evaluated with the Mouse IL-10 ELISA. The results of these two experiments are reported in the table below.

In Vivo LPS-Stimulated Serum Samples		LPS-Stimulated Splenocyte Supernatants	
Time After Injection (min)	**Mouse IL-10** (pg/mL)	**Days After Stimulation**	**Mouse IL-10** (pg/mL)
0	30	1	79
60	1170	2	277
90	4225	3	852
120	1623		
150	1064	**Non-stimulated Control**	
180	497	3	0

***Note:** Strain-to-strain differences in the base levels of mouse IL-10 and nonspecific activity (up to 45pg/mL) were observed when testing commercially available normal serum samples in this assay. For best results, test appropriate control mouse samples in every experiment.

Linearity of Dilution

Dilution linearity was determined by serially diluting 10 different positive samples. The dilutions were evaluated in the ELISA and "found" doses are plotted against the "expected" doses. An "r" value and slope of the regression line close to 1 indicate that the samples dilute linearly.



Cited Reference

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

Data Templates

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

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