INSTRUCTIONS

Rat IL-10 ELISA Kit

ERIL10 ERIL102 ERIL105

1415.5

Number	Description
ERIL10	**Rat Interleukin-10 (IL-10) ELISA Kit,** sufficient reagents for 96 determinations
ERIL102	**Rat IL-10 ELISA Kit,** sufficient reagents for 2 × 96 determinations
ERIL105	**Rat IL-10 ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	ERIL10	ERIL102	ERIL105
Anti-Rat IL-10 Pre-coated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Rat IL-10 Standard	2 vials	4 vials	10 vials
Standard Diluent, contains 0.1% NaN_3	20mL	2 × 20mL	5 × 20mL
Biotinylated Antibody Reagent, contains 0.1% NaN_3	11mL	2 × 11mL	5 × 11mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
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

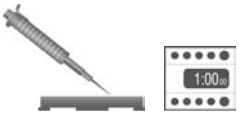
The Thermo Scientific™ Rat IL-10 ELISA Kit is for measuring rat IL-10 in culture supernatants; EDTA, heparin and sodium citrate plasma; and serum.

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 1 hour.

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 Premixed TMB Substrate Solution to each well.

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

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

12. Measure absorbance on a plate reader at 450nm minus 550nm. Calculate results using graph paper or 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 2L container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well 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 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 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.
- Standard Diluent must be used when diluting all samples.
- 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 kit components.
- 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.
- Some components of this kit contain sodium azide. Please dispose of reagents according to local regulations.

Additional Precaution for the 2-plate and 5-plate Kits

- 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

Sample Handling

- Serum; EDTA, heparin or sodium citrate plasma; or culture supernatants may be tested in this assay.
- 100µL per well of cell culture supernatant is required in this assay. For serum and plasma samples, 100µL per well of a 1:2 sample dilution 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 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 rat IL-10 concentration possibly exceeds the highest point of the standard curve (i.e., 500pg/mL) prepare one or more five-fold dilutions of the test sample. For example, a five-fold dilution is prepared by adding 50µL of test sample to 200µL of Standard Diluent. Mix thoroughly between dilutions before assaying. Prepare all sample dilutions using the Standard Diluent provided.

Reagent Preparation

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

Wash Buffer

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 bottle (50mL) to the two-liter container and dilute to a final volume of 1.5L with ultrapure water. Mix 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.

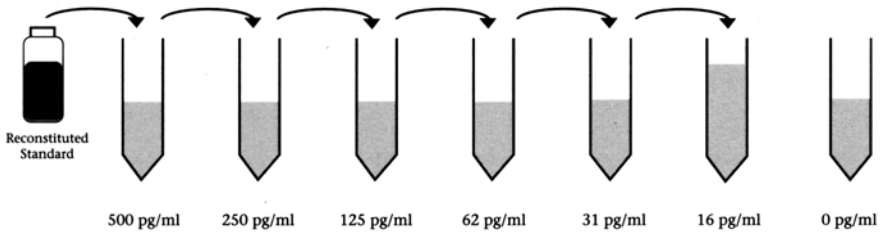
Standards

- **(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. Reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. Mix by gently inverting the vial until the contents have completely dissolved. Use the Standard Diluent provided to prepare the standard curve serial dilutions.

2. Label seven tubes, one for each standard curve point: 500, 250, 125, 62, 31, 16, and 0pg/mL, then prepare 1:2 serial dilutions for the standard curve as follows:
3. Pipette 250µL of Standard Diluent into each tube.
4. Pipette 250µL of the reconstituted standard into the first tube (i.e., 500pg/ml) and mix.
5. Pipette 250µL of this dilution into the next tube (i.e., 250pg/mL) and mix.
6. Repeat serial dilutions (using 250µL) four 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 zero standard (blank or negative control), rat IL-10 standards and test samples. Perform six standard points and one blank in duplicate with each series of unknown samples. Serum and plasma samples must be diluted 1:2 with Standard Diluent before performing the assay.

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

   Note: If the rat IL-10 concentration in any sample possibly exceeds the highest point on the standard curve, 500pg/mL, see Sample Preparation – Sample Dilution 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 1 hour at room temperature, 20-25°C.
3. Carefully remove adhesive plate cover and wash plate THREE times with Wash Buffer as described in the Plate Washing Section (Section B).

B. Plate Washing

Note: Automated plate washers may produce sub-optimal results. For best results, perform a manual wash the first time the assay is performed. Automated plate washing may require validation to determine the number of washes necessary to achieve optimal results; typically, 3-5 washes are sufficient.

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 wash 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 with Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material. The number of washes necessary may require optimization.

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.

1. Add 100µL of Biotinylated Antibody Reagent to each well containing sample or standard.

2. Carefully attach a new adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over edges and down each strip. Incubate plate for 1 hour at room temperature, 20-25°C.
3. Carefully remove the adhesive plate cover and wash THREE times with Wash Buffer as described in the Plate Washing Section.

D. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution **immediately before use.** Do not prepare more Streptavidin-HRP 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 a 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 adhesive plate cover 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 100µL of Stop Solution to each well.

F. Absorbance Measurement

Note: Evaluate the plate within 30 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 450 nm only.

Note: When the 550 nm measurement is omitted, absorbance values will be higher.

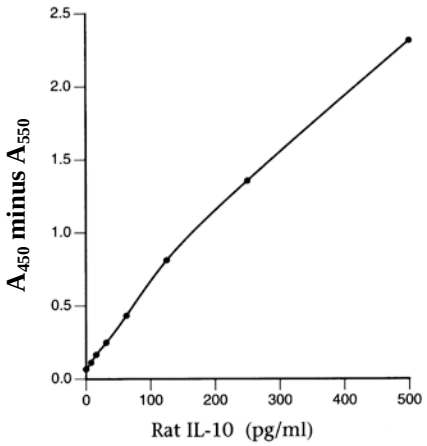
G. Calculation of Results

- The standard curve is used to determine rat 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 rat IL-10 concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. The rat IL-10 amount in each sample is determined by interpolating from the absorbance value (Y-axis) to the rat IL-10 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 rat IL-10 in the sample. Because serum and plasma samples were diluted 1:2 before performing the assay, multiply values interpolated from the standard curve by two to calculate the rat IL-10 amount in these samples.

  Note: The standard curve illustrated here uses point-to-point data reduction.
- 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%.

Standard Curve Example



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: 16-500pg/mL
Suggested standard curve points are 500, 250, 125, 62, 31, 16, and 0pg/mL.

Reproducibility: Reproducibility of the Rat IL-10 ELISA is evaluated in each sample matrix. To determine intra-assay precision, 20 replicates of samples containing two levels of recombinant rat IL-10 are evaluated on a single plate.
To evaluate inter-assay precision, the same or additional samples are tested by three operators who perform at least three separate assays on more than one day. Ten duplicate sample values are used to calculate inter-assay precision data for each level of rat IL-10. Results are summarized in Table 1.

Table 1. Intra-assay and inter-assay precision of the Rat IL-10 ELISA Kit.

		Intra-assay Precision			Inter-assay Precision		
Sample	**Level**	**Mean (pg/mL)**	**SD (pg/mL)**	**CV (pg/mL)**	**Mean (pg/mL)**	**SD (pg/mL)**	**CV (pg/mL)**
Serum	1	80.9	4.4	5.5	80.9	8.1	10.1
	2	239.9	14.9	6.2	238.6	23.1	9.7
EDTA Plasma	1	116.9	3.5	3.0	131.9	14.5	10.9
	2	397.9	32.6	8.2	389.1	37.4	9.6
Citrate Plasma	1	116.9	4.8	4.1	127.5	14.6	11.4
	2	385.5	20.2	5.2	391.1	22.8	5.8
Heparin Plasma	1	98.1	3.2	3.3	102.8	9.4	9.2
	2	353.3	15.6	4.4	350.3	23.1	6.6
Cell Culture Supernatant	1	139.5	12.5	8.9	150.5	13.5	8.9
	2	484.4	19.4	4.0	497.3	16.8	3.4

Specificity: This ELISA is specific for the measurement of natural and recombinant rat IL-10. This ELISA does not cross-react with the following cytokines: rat IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-6, MCP-1, MIP-1α, RANTES, or TNFα. No cross-reactivity is observed to human or mouse IL-10. All cross-reactants are evaluated at 20ng/mL.

Calibration: The standard in this ELISA is calibrated to an internal rat IL-10 reference standard.

Expected Values: Ten serum and plasma samples collected from apparently healthy rats are evaluated in this assay. The levels of rat IL-10 found in each sample type are reported in Table 2.

Table 2. Rat IL-10 levels from apparently healthy rats.

Sample Type	Average	Range
Serum	15.8pg/mL	5.7-45.7pg/mL
EDTA Plasma	15.0pg/mL	11.4-21.0pg/mL
Citrate Plasma	9.3pg/mL	5.3-14.0pg/mL
Heparin Plasma	11.4pg/mL	5.7-30.0pg/mL

Spike and Recovery: Recovery of rat IL-10 is evaluated using the Rat IL-10 ELISA Kit. Individual serum and plasma samples and Standard Diluent controls are spiked with recombinant rat IL-10. Endogenous rat IL-10 levels are determined by evaluating non-spiked samples along with spiked aliquots of the same samples in the ELISA. Expected values are calculated by adding endogenous rat IL-10 levels to those of spiked diluent controls. Percent (%) recovery is determined by dividing observed by expected values. Results for representative individual samples and populations are summarized in Table 3.

Table 3. Spike and recovery values from representative individual samples and a sample population.

	Representative Samples			Sample Population			
Sample	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)	n
Serum	477.9	452.3	94.6	488.4	474.9	97.2	6
EDTA Plasma	466.8	426.5	91.4	465.9	424.7	91.2	6
Citrate Plasma	475.6	391.2	82.3	476.0	394.6	82.9	6
Heparin	473.5	406.2	85.8	473.2	401.3	84.8	6

Linearity of Dilution: Serum, plasma and cell culture supernatant samples containing rat IL-10 are serially diluted in Standard Diluent and evaluated in the Rat IL-10 ELISA. Observed values are compared to expected values to calculate percent (%) recovery and demonstrate the dilution linearity of the assay (Table 4).

Table 4. Dilution linearity of the Rat IL-10 ELISA Kit.

Sample	Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Serum	Neat	495.1	321.1	-
	1:2	247.5	151.6	94.4
	1:4	123.8	83.4	103.8
	1:8	61.9	41.3	103.0
	1:16	30.9	23.0	114.4
EDTA Plasma	Neat	270.5	270.5	-
	1:2	135.2	141.3	104.5
	1:4	67.6	62.0	91.7
	1:8	33.8	30.0	88.7
	1:16	16.9	14.3	84.6
Cell Culture Supernatant	Neat	379.6	379.6	-
	1:2	189.8	206.2	108.6
	1:4	94.9	102.4	107.9
	1:8	47.5	45.5	95.9

Cited Reference

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

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Data Templates

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