# RayBio® Mouse IL-3 ELISA Kit

Catalog #: ELM-IL3

User Manual
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Caution:
Extraordinarily useful information enclosed

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Empowering your proteomics

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RayBiotech, Inc.

# RayBio® Mouse IL-3 ELISA Kit Protocol

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Please read the entire manual carefully before starting your experiment

## I. INTRODUCTION

The RayBio® Mouse IL-3 ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse IL-3 in serum, plasma and cell culture supernatants. This assay employs an antibody specific for mouse IL-3 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-3 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse IL-3 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-3 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

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## II. STORAGE

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

## III. REAGENTS

| **Component** | **Size / Description** | **Storage / Stability After Preparation** |
|---|---|---|
| IL-3 Microplate (Item A) | 96 wells (12 strips x 8 wells) coated with anti-Mouse IL-3. | 1 month at 4°C* |
| Wash Buffer Concentrate (20X) (Item B) | 25 ml of 20X concentrated solution. | 1 month at 4°C |
| Standard Protein (Item C) | 2 vials of Mouse IL-3. 1 vial is enough to run each standard in duplicate. | 1 week at -80°C |
| Detection Antibody IL-3 (Item F) | 2 vials of biotinylated anti-Mouse IL-3. Each vial is enough to assay half the microplate. | 5 days at 4°C |
| HRP-Streptavidin Concentrate (Item G) | 200 µl 300X concentrated HRP-conjugated streptavidin. | Do not store and reuse. |
| TMB One-Step Substrate Reagent (Item H) | 12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution. | N/A |
| Stop Solution (Item I) | 8 ml of 0.2 M sulfuric acid. | N/A |
| Assay Diluent D (Item K) | 15 ml of 5X concentrated buffer. | 1 month at 4°C |
| Assay Diluent B (Item E) | 15 ml of 5X concentrated buffer. | 1 month at 4°C |

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

## IV. ADDITIONAL MATERIALS REQUIRED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

## V. REAGENT PREPARATION

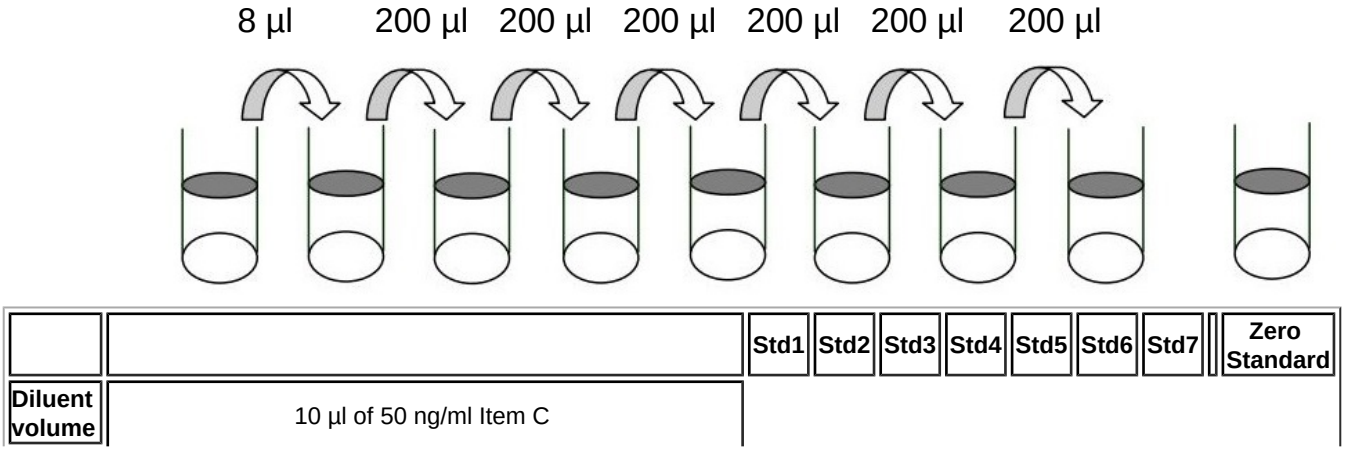
1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. Assay Diluent D (Item K) and Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.

3. Sample dilution: 1X Assay Diluent D (Item K) should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2 fold.

   **Note:** Levels of IL-3 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Briefly spin a vial of Item C. Add 400 µl 1X Assay Diluent D (Item K) into Item C vial to prepare a 50 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 10 µl of IL-3 standard from the vial of Item C, into a tube with 90 µl 1X Assay Diluent D to prepare a 5,000 pg/ml standard solution. Add 8 µl 5,000 pg/ml of IL-3 standard solution into a tube with 992 µl 1X Assay Diluent D to prepare a 40 pg/ml standard solution. Pipette 300 µl 1X Assay Diluent D into each tube. Use the 40 pg/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent D serves as the zero standard (0 pg/ml).



1. If the Wash Concentrate (20X) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

2. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1X Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC

## VIII. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

### A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve

## C. SPIKING & RECOVERY

Recovery was determined by spiking various levels of Mouse IL-3 into the sample types listed below. Mean recoveries are as follows:

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| Serum | 104.3 | 73-133 |
| Plasma | 113.9 | 65-162 |
| Cell culture media | 102.4 | 66-132 |

## D. LINEARITY

| Sample Type | | Serum | Plasma | Cell Culture Media |
|---|---|---|---|---|
| 1:2 | Average % of Expected | 89.7 | 100.7 | 84.19 |
| | Range (%) | 92-132 | 89-114 | 73-92 |
| 1:4 | Average % of Expected | 100.5 | 77.69 | 76.33 |
| | Range (%) | 99-108 | 47-85 | 67-85 |

**E. REPRODUCIBILITY**

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

**IX. SPECIFICITY**

This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin, Eotaxin-2, Fas Ligand, Fractalkine, GCSF, IFN-gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1 beta, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-5, M-

## X. TROUBLESHOOTING GUIDE

| Problem | Cause | Solution |
|---|---|---|
| Poor standard curve | ○ Inaccurate pipetting<br>○ Improper standard dilution | ○ Check pipettes<br>○ Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing |
| Low signal | ○ Improper preparation of standard and/or biotinylated antibody<br>○ Too brief incubation times<br>○ Inadequate reagent volumes or improper dilution | ○ Briefly spin down vials before opening. Dissolve the powder thoroughly.<br>○ Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).<br>○ Check pipettes and ensure correct preparation |
| Large CV | ○ Inaccurate pipetting<br>○ Air bubbles in wells | ○ Check pipettes<br>○ Remove bubbles in wells |
| High background | ○ Plate is insufficiently washed<br>○ Contaminated wash buffer | ○ Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.<br>○ Make fresh wash buffer |

| | | |
|---|---|---|
| Low sensitivity | ○ Improper storage of the ELISA kit<br>○ Stop solution | ○ Store your standard at <-70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light.<br>○ Add stop solution to each well before reading plate |

RayBio®

ELISA Kits

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This product is for research use only.