User Guide

# Cotton rat IL-4 ELISA Kit

For Cell Culture Media

**RAB0582**

## Introduction

The Cotton rat IL-4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IL-4 in cell culture supernatants (serum/plasma have not been validated yet in this assay). This assay employs an antibody specific for human IL-4 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-4 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IL-4 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-4 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

## Storage

The entire kit may be stored at -20°C for up to 1 year from 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.

## Components

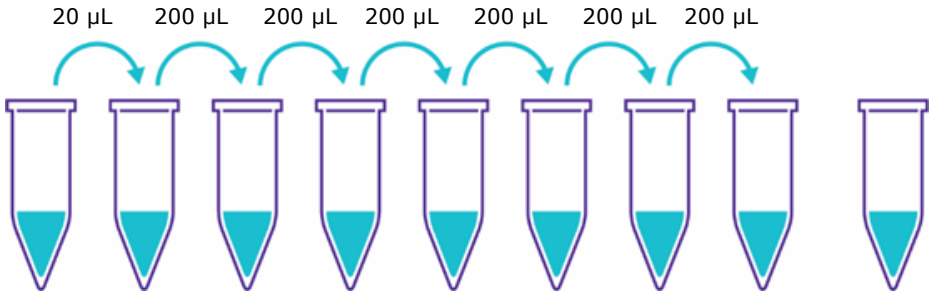
- Cotton rat IL-4 Microplate - RAB0582A-1EA-KC: 96 wells (12 strips x 8 wells) coated with anti-Cotton rat IL-4. Stable in storage 1 month at 4 °C once opened. Return unused wells to the pouch containing desiccant pack, reseal along the edge.
- Cotton rat IL-4 Standard Protein - RAB0582C-1VL-KC: 2 vials of Cotton rat IL-4. 1 vial is enough to run each standard in duplicate. Stable in storage 1 week at -80 °C once opened.
- Cotton rat IL-4 Detection Antibody - RAB0582F-1VL-KC: 2 vials of biotinylated anti-Cotton rat IL-4. Each vial is enough to assay half the microplate. Stable in storage 5 days at 4 °C once opened.
- Wash Buffer - RABWASH4: 25 mL of 20X concentrated solution. Stable in storage 1 month at 4 °C once opened.
- HRP-Streptavidin - RABHRP5: 200 µL 700X concentrated HRP-conjugated streptavidin. Do not store and reuse.
- TMB One-Step Substrate Reagent - RABTMB3: 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
- Stop Solution - RABSTOP3: 8 mL of 0.2 M sulfuric acid.
- 5X Assay Diluent B - RABELADE-15ML: 15 mL of 5X concentrated buffer. Stable in storage 1 month at 4°C once opened.

## Additional Materials Required (But Not Provided)

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

## Reagent Preparation

1. Bring all reagents and samples to room temperature (18-25 °C) before use.
2. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: 1X Assay Diluent B should be used for dilution of cell culture supernatants.
   **Note:** Levels of IL-4 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 Standard Protein. Add 400 µL 1x Assay Diluent B (should be diluted 5-fold with deionized or distilled water before use) into the Standard Protein vial to prepare a 25 ng/mL standard solution. Dissolve the powder thoroughly by a gentle mix. Add 20 µL of the IL-4 standard solution from the vial of Standard Protein, into a tube with 605 µL 1X Assay Diluent to prepare a 800 pg/mL standard solution. Pipette 300 µL 1x Assay Diluent B into each tube. Use the 800 pg/mL standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent B serves as the zero standard (0 pg/mL).




| | | Std 1 | Std 2 | Std 3 | Std 4 | Std 5 | Std 6 | Std 7 | 0 Std |
|---|---|---|---|---|---|---|---|---|---|
| **Diluent volume** | Std + 400 µL | 605 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL |
| **Concentration** | 25 ng/mL | 800 pg/mL | 320 pg/mL | 128 pg/mL | 51.20 pg/mL | 20.48 pg/mL | 8.192 pg/mL | 3.277 pg/mL | 0 pg/mL |

5. If the Wash Buffer (20X) 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.
6. Briefly spin the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in step 5 of the assay procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 700-fold with 1X Assay Diluent B.

   For example: Briefly spin the HRP-Streptavidin vial and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin concentrate into a tube with 14 mL 1X Assay Diluent B to prepare a 700-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

## Assay Procedure

1. Bring all reagents and samples to room temperature (18-25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

## Assay Procedure Summary

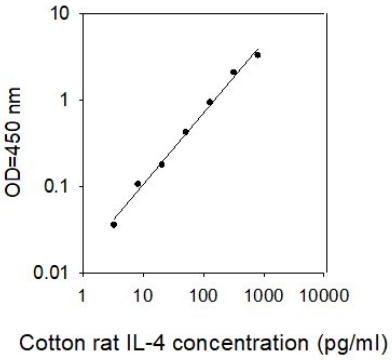
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature.
3. Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

## 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.

## Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.




## Sensitivity

The minimum detectable dose of Cotton rat IL-4 was determined to be 2.65 pg/mL.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

## Spiking & Recovery

Recovery was determined by spiking various levels of Cotton rat IL-4 into the sample types listed below. Mean recoveries are as follows:

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| Cell Culture Media | 98 | 91-103 |

## Linearity

| Sample Type | | Cell Culture Media |
|---|---|---|
| 1:2 | Average % of Expected | 98 |
| | Range (%) | 85-112 |
| 1:4 | Average % of Expected Range | 87 |
| | Range (%) | 72-101 |

## Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

## Specificity

This ELISA antibody pair detects Cotton rat IL-4. Other species not determined.

## Troubleshooting Guide

| Problem | Cause | Solution |
|---|---|---|
| Poor standard curve | Inaccurate pipetting | Check pipettes |
| | Improper standard dilution | Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing |
| Low signal | Improper preparation of standard and/or biotinylated antibody | Briefly spin down vials before opening. Dissolve the powder thoroughly. |
| | Too brief incubation times | Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4 °C with gentle shaking.<br><br>Note: may increase overall signals including background. |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| Large CV | Inaccurate pipetting | Check pipettes |
| | Air bubblies in wells | Remove bubbles in wells |
| High background | Plate is insufficiently washed | Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. |
| | Contaminated wash buffer | Make fresh wash buffer |
| Low sensitivity | Improper storage of the ELISA kit | Store your standard at -70 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light. |
| | Stop Solution | Add stop solution to each well before reading plate |

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