Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–500 pg/mL Rt IL-4.

Standard Rt IL-4 (pg/mL)	Optical Density (450 nm)
500	2.309
250	1.388
125	0.742
62.5	0.467
31.2	0.295
15.6	0.209
7.8	0.168
0	0.118

Intra-assay precision

Samples of known Rt IL-4 concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	72.8	153.4	308.7
SD	5.7	9.9	19.5
%CV	7.8	6.5	6.3

SD = Standard Deviation; CV = Coefficient of Variation

Linearity of dilution

Rat serum containing 324 pg/mL of measured Rt IL-4 was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	75.1	154.8	317.9
SD	6.6	12.7	26.0
%CV	8.8	8.2	8.2

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recoveries of Rt IL-4 added to rat serum and tissue culture media containing fetal bovine serum were measured with the Rt IL-4 ELISA Kit.

Sample	Average % Recovery
Serum	93
Tissue culture medium +1% fetal bovine serum	118
Tissue culture medium +10% fetal bovine serum	114

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Rt IL-4 kit and found to have no cross-reactivity: **Human** IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15; **Mouse** IL-1β, IL-2, IL-4, IL-6, IFN-γ, TNF-α; **Rat** MCP-1, MIP-2,TNF-α

Sensitivity

The minimum detectable dose of Rt IL-4 is <2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

For support visit www.lifetechnologies.com/support or contact **techsupport@lifetech.com**.

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04 February 2015

novex life technologies

Rat IL-4 ELISA Kit

Catalog no. KRC0041 **Quantity:** 96 tests

Pub. No. MAN0011081 **Rev** 2.0

Description

The Rat IL-4 ELISA Kit is a solid-phase sandwich Enzyme Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of rat IL-4 in rat serum, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant rat IL-4.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	96 Test Kit
Rt IL-4 Antibody Coated Wells. 96 well plate.	1 plate
Rt IL-4 Biotin Conjugate. Contains 0.1% sodium azide.	6 mL
Rt IL-4 Standard. Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Wash Buffer Concentrate (25X).	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL
Streptavidin-HRP (100X). Contains 3.3 mM thymol.	0.125 mL
Streptavidin-HRP Diluent. Contains 3.3 mM thymol.	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL
Stop Solution.	25 mL
Adhesive Plate Covers.	3

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **www.lifetechnologies.com/manuals** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

For Research Use Only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 20 mL of Wash Buffer Concentrate (20X) with 380 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

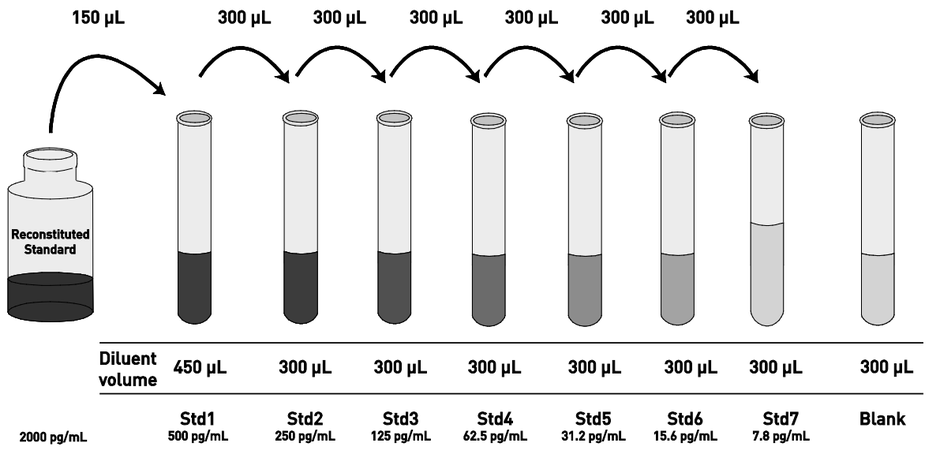
Dilute samples

- Dilute samples >500 pg/mL in Standard Diluent Buffer.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Rt IL-4 Standard to 2000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 2000 pg/mL Rt IL-4.
   Use the standard within 1 hour of reconstitution.
2. Add 150 µL Reconstituted Standard to one tube containing 450 µL Standard Diluent Buffer and label as 500 pg/mL Rt IL-4.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 250, 125, 62.5, 31.2, 15.6, 7.8, and 0 pg/mL Rt IL-4.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare Streptavidin-HRP solution

Note: Prepare Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3 hours.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.

Bind antigen

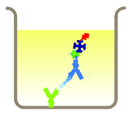
1. Add 100 µL of standards and background controls into the appropriate wells.
2. Add 100 µL of **serum/plasma** samples into the appropriate wells.
   OR
   Add 50 µL of Standard Diluent Buffer to sample wells, followed by 50 µL of **cell culture supernatant/buffered solution** or control samples (see page 2) in the appropriate wells.
3. Add 50 µL Rt IL-4 Biotin Conjugate solution into each well except the chromogen blanks.
4. Cover the plate with plate cover and incubate for 2 hours at at room temperature.
5. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Streptavidin-HRP

6. Add 100 µL Streptavidin-HRP (see page 2) into each well except the chromogen blanks.
7. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
8. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

Add chromogen

9. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
10. Cover the plate with plate cover and incubate for 30 minutes at room temperature **in the dark**.
    Note: TMB should not touch aluminum foil or other metals.



Add stop solution

11. Add 100 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
   Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.