Quantikine® ELISA

Rat IL-4 Immunoassay

Catalog Number R4000

For the quantitative determination of rat Interleukin 4 (IL-4) concentrations in cell culture supernates and serum.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
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DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
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24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
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INTRODUCTION

Interleukin 4 (IL-4) is a pleiotropic cytokine that has multiple immune response-modulating activities on a variety of cell types (1). IL-4 is a B cell activation/differentiation factor that regulates Ig isotype switching, particularly IgG_1 and IgE (1). It suppresses the development of IFN-γ-producing $CD4^+$ T cells and regulates the differentiation of naïve precursor T helper cells to the Th2 subset that mediates allergic and humoral immune response (2, 3). Together with TNF-α, IL-4 synergistically induces the expression of VCAM-1 on vascular endothelial and smooth muscle cells, resulting in the selective recruitment of eosinophils and lymphocytes to the site of inflammation (4, 5). IL-4 downregulates the production of inflammatory mediators such as IL-1, TNF-α, and PGE_2 in monocytes (1). IL-4 has also been shown to have anti-tumor activity both *in vivo* and *in vitro* (2). Cells that have been shown to secrete IL-4 include basophils (6), $CD8^+$ T cells (7), $CD4^+$ memory and naïve Th2 cells (8, 9), mast cells (10), eosinophils (11), and virally activated dendritic cells (12).

The cDNA sequence of rat IL-4 predicts a 147 amino acid (aa) residue precursor protein containing a 24 aa residue signal peptide that is cleaved to generate the 123 aa residue mature protein containing four potential N-linked glycosylation sites and seven cysteine residues (13-15). Six of the seven cysteine residues are involved in the formation of three intramolecular disulfide linkages that are essential for activity (13). In humans, an alternately spliced truncated IL-4 variant lacking the sequence encoded by the second of the four exons has been described (16-18). Recombinant human truncated IL-4 has been shown to have antagonistic effects on IL-4 activities in human monocytes and B cells. It is not known if truncated IL-4 is produced naturally (18). Mature rat IL-4 shares 60% and 44% amino acid sequence similarity with mouse and human IL-4, respectively (14, 15).

Functional IL-4 receptor complexes are present in many cell types including astrocytes, fibroblasts, hepatocytes, hematopoietic, epithelial, endothelial, and vascular smooth muscle cells (3). One type of IL-4 receptor complex consists of a heterodimeric complex between the 140 kDa IL-4 Rα chain that binds IL-4 with high affinity, and the gamma common chain (γc) which is required for IL-4 signaling (3). In non-hematopoietic cells where γc is absent, IL-4 Rα can also heterodimerize with the 65 kDa IL-13 Rα1 chain to transduce IL-4 signals (3). In some cells, IL-4 signaling as a result of IL-4 Rα homodimerization has also been reported (3). In addition to the membrane bound form of IL-4 Rα, a naturally occurring soluble form of IL-4 Rα has been identified in mouse biological fluids and in mouse cell culture supernatants (19-21).

The Quantikine® Rat IL-4 Immunoassay is a 4.5 hour solid phase ELISA designed to measure rat IL-4 levels in cell culture supernates and serum. It contains *E. coli*-expressed recombinant rat IL-4 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant rat IL-4 accurately. Results obtained using natural rat IL-4 showed dose response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural rat IL-4.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat IL-4 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-4 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat IL-4 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-4 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Rat IL-4 Microplate	890737	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for rat IL-4.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Rat IL-4 Standard	890738	Recombinant rat IL-4 in a buffered protein base, with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Rat IL-4 Control	890740	Recombinant rat IL-4 in a buffered protein base with preservatives; lyophilized. The assayed value of the control should be within the range specified on the label.	
Rat IL-4 Conjugate	890739	12 mL of a polyclonal antibody specific for rat IL-4 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	21 mL of buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

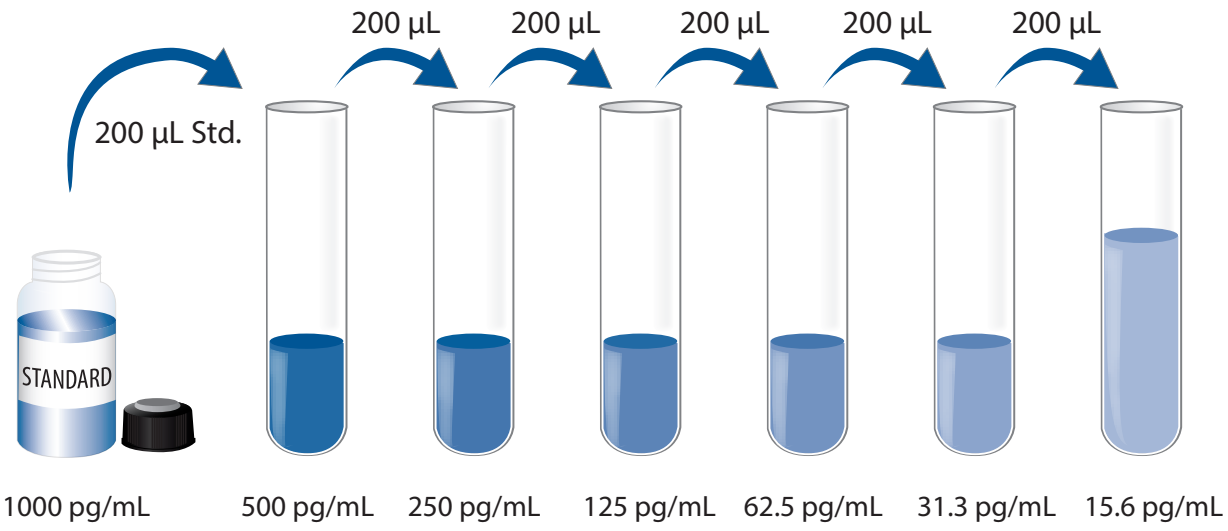
Rat IL-4 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Rat IL-4 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat IL-4 Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-16 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Rat IL-4 Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD5-16 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare reagents, standard dilutions, and control as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control, or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, 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.
6. Add 100 µL of Rat IL-4 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

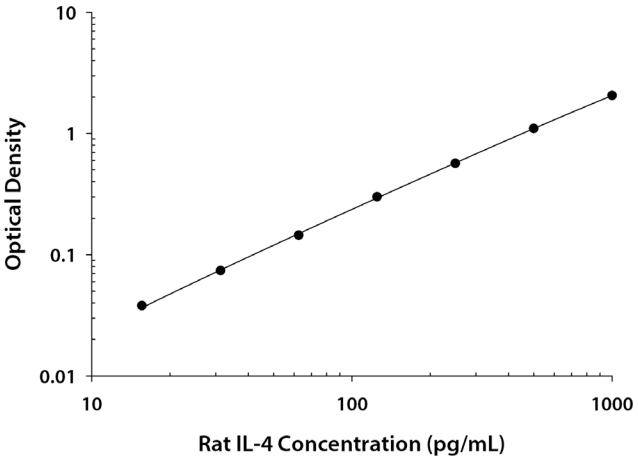
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the rat IL-4 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.038	0.037	—
15.6	0.074 0.076	0.075	0.038
31.3	0.111 0.111	0.111	0.074
62.5	0.182 0.183	0.182	0.145
125	0.331 0.344	0.338	0.301
250	0.599 0.611	0.605	0.568
500	1.130 1.143	1.136	1.099
1000	2.088 2.102	2.095	2.058

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	48.2	111	721	47.7	112	742
Standard deviation	2.5	4.9	23.6	3.9	6.1	31.8
CV (%)	5.2	4.4	3.3	8.2	5.4	4.3

RECOVERY

The recovery of rat IL-4 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	110	104-117%
Serum (n=7)	94	88-101%

LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with various concentrations of rat IL-4 in each matrix were diluted with calibrator diluent and then assayed. Results from typical sample dilutions are shown.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}}$ x 100
Cell culture supernates	Neat	785	———	———
	1:2	389	392	99%
	1:4	199	196	102%
	1:8	100	98	102%
	1:16	52	49	106%
Serum	Spiked	695	———	———
	1:2	353	348	101%
	1:4	184	174	106%
	1:8	89	87	102%
	1:16	46	44	105%

SENSITIVITY

The minimum detectable dose (MDD) of rat IL-4 is typically less than 5 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant rat IL-4 produced at R&D Systems®.

SAMPLE VALUES

Serum - Forty samples were evaluated for detectable levels of rat IL-4 in this assay. All samples measured less than the lowest standard, 15.6 pg/mL.

Cell Culture Supernates - Rat thymus cells (1 x 10^7 cells/mL) from Lewis rats were cultured for 5 days in DMEM containing 10% fetal bovine serum and 50 ng/mL of recombinant rat IL-2 and stimulated with 5 µg/mL of PHA, followed by replacement of the media containing 50 ng/mL of PMA plus 500 ng/mL of calcium ionomycin and cultured for 48 hours. An aliquot of the cell culture supernate was removed, assayed for rat IL-4, and measured 270 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat IL-4.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range rat IL-4 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:

CINC-1
GDNF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-6
IL-10
IL-18
β-NGF
PDGF-BB
TNF-α

Recombinant mouse:

IL-4

Recombinant human:

IL-4
IL-4 R

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