LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of IL-4 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	94	103
	Range(%)	86—105	95—112
1:4	Average% of Expected	92	105
	Range(%)	85—101	96—116
1:8	Average% of Expected	96	104
	Range(%)	88—104	97—111
1:16	Average% of Expected	95	107
	Range(%)	85—104	99—116

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Rat IL-4 Immunoassay

Catalog Number: SEKR-0004

For the quantitative determination of Rat interleukin-4 (IL-4) concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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Performance Characteristics

SENSITIVITY: The minimum detectable dose was 3.9 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant rat IL-4 . The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant rat	Recombinant Mouse	Recombinant human
IL-1a		IL-4
IL-2		IL-4R
IL-6		
IL-10		
IL-18		
IFMN-γ		
TNF-α		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of IL-4 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of IL-4 in two matrices

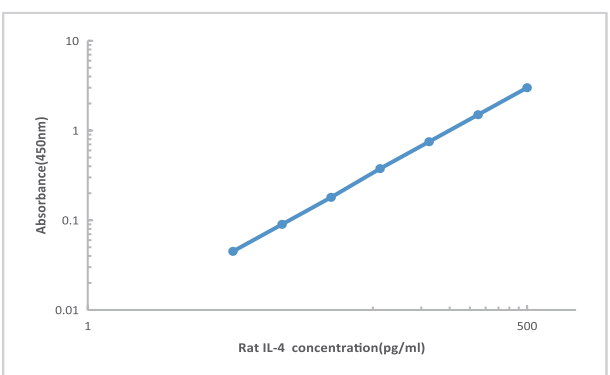
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	96	88—105
Cell culture supernatants	98	89—108

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.

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

Typical data using the IL-4 ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.056	0.058	0.057	---
7.8	0.093	0.098	0.095	0.038
15.625	0.174	0.180	0.177	0.120
31.25	0.321	0.345	0.333	0.276
62.5	0.486	0.512	0.499	0.442
125	0.769	0.735	0.752	0.695
250	1.326	1.351	1.338	1.281
500	2.436	2.472	2.454	2.397



Representative standard curve for IL-4 ELISA

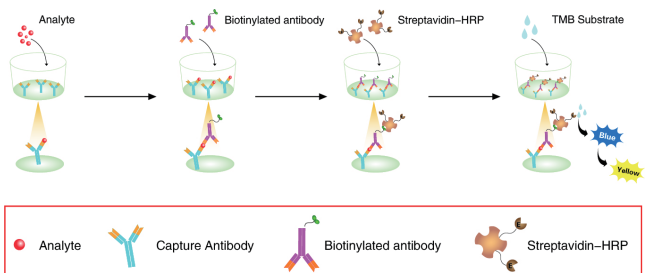
BACKGROUND

Interleukin 4 (IL-4) is a pleiotropic cytokine that has multiple immune response-modulating activities on a variety of cell types. IL-4 is a B cell activation/differentiation factor that regulates Ig isotype switching, particularly IgG1 and IgE. It suppresses the development of IFN-γ-producing CD4+ T cells and regulates the differentiation of naive precursor T helper cells to the Th2 subset that mediates allergic and humoral immune response. 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. IL-4 downregulates the production of inflammatory mediators such as IL-1, TNF-α, and PGE2 in monocytes.

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 a residue mature protein containing four potential N-linked glycosylation sites and seven cysteine residues. Six of the seven cysteine residues are involved in the formation of three intramolecular disulfide linkages that are essential for activity. In humans, an alternately spliced truncated IL-4 variant lacking the sequence encoded by the second of the four exons has been described. 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. Mature rat IL-4 shares 60% and 44% amino acid sequence similarity with mouse and human IL-4, respectively.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells; any IL-4 present is captured by the coated antibody after incubation. After washing away any unbound substances, a biotin-conjugate antibody specific for IL-4 is added to detect the captured IL-4 protein in the sample. Following a wash to remove any unbound combination, horseradish peroxidase (HRP)-conjugated Streptavidin is added to the wells. After extensive washing, a tetramethyl-benzidine (TMB) reagent is added to the wells for signal development. Solution containing sulfuric acid is used to stop color development. The color intensity, proportional to the quantity of bound protein, is then measurable at 450nm.



ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, incubate 90 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-rat IL-4 antibody to each well, incubate 60 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃.

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 15 minutes,37℃. Protect from light.

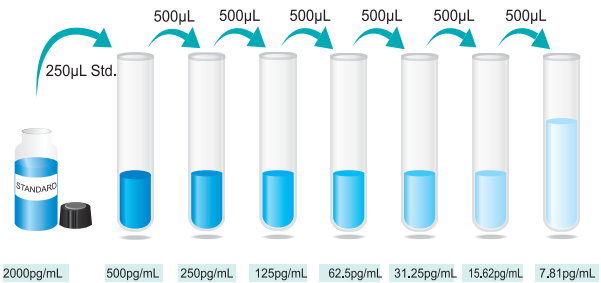
⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the IL-4 concentrations versus the log of the O.D. and the best fit line can be determined by

of 2000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 750uL of Standard/Sample Diluent into 500pg/ml tube and the remaining tubes. Use the stock solution of 500pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 500 pg/mL standard serves as the high standard. The Standard/ Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of IL-4 standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-rat IL-4 antibody**:Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**

TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	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℃**
Standard - lyophilized,2000 pg/ml upon reconstitution	2 vials	Aliquot and Store at 2-8°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

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

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard/Specimen** -Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution