ELISA Kit

Catalog # KAC1281 (96 tests)

Human
Interleukin 4 (IL-4)

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Contents and Storage

Storage

Store at 2 to 8°C.

Contents

Reagents Provided	96 Test Kit
Standard 0 in human serum, with preservatives (benzamidin and thymol). Lyophilized. Refer to vial label for quantity and reconstitute with 5 mL distilled water.	1 vial
Standards 1 - 5 in human plasma, with preservatives (benzamidin and thymol). Lyophilized. Refer to vial label for concentration and reconstitute with 1 mL distilled water. Note: 1 pg of the standard preparation is equivalent to 10 mU NIBSC 88/656.	5 vials
Controls 1 and 2 in human serum, with thymol. Lyophilized. Refer to vial label for range. Reconstitute with 1 mL distilled water.	2 vials
Specimen Diluent (human serum with benzamidin and thymol). Lyophilized. Refer to vial label for reconstitution volume.	2 vials
Incubation Buffer (Phosphate buffer with BSA and thymol). 11 mL per bottle.	1 bottle
Anti-IL-4 Antibody-Coated Wells, 96 wells per plate.	1 plate
Anti-IL-4-HRP Conjugate in buffered solution with bovine serum albumin and thymol; 6 mL per bottle.	1 bottle
Wash Buffer Concentrate (200X); 10 mL per bottle.	1 bottle
Concentrated Chromogen, Tetramethylbenzidine (TMB) in DMF, 1 mL per vial.	1 vial
Substrate Buffer: H_2O_2 in acetate/citrate buffer; 21 mL per botttle.	3 bottles
Stop Solution: H_2SO_4, 1.8 N, 6 mL per bottle.	1 bottle

Disposal Note

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.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Avoid any skin contact with Stop Solution (H_2SO_4) and Concentrated Chromogen (TMB), Substrate Buffer, and Chromogenic Solution. In case of contact wash thoroughly with water.

Introduction

Purpose

An immunoassay for the quantitative measurement of human interleukin 4 (IL-4) in serum.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Invitrogen IL-4 ELISA is a solid phase Enzyme Linked Immunosorbent Assay performed on a microtiter plate. The assay is based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of IL-4 are used. The use of a number of distinct MAbs avoids hyperspecificity and allows high sensitive assays with extended standard range and short incubation time. Standards or samples containing IL-4 react with capture monoclonal antibodies (MAbs 1) coated on the microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAbs 1 - IL-4 - MAb 2 - HRP, the microtiter plate is washed to remove unbound enzyme labelled antibodies.

Bound enzyme- labelled antibodies are measured through a chromogenic reaction. Chromogenic solution (TMB+H_2O_2) is added and incubated. The reaction is stopped with the addition of Stop Solution (H_2SO_4) and the microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is proportional to the IL-4 concentration. A standard curve is plotted and IL-4 concentration in a sample is determined by interpolation from the standard curve.

Background Information

Interleukin-4 (IL-4) is a 15-19 kDa glycoprotein produced by the TH2 sub-type of CD4+ T-lymphocytes and by mast cell precursors. IL-4 down regulates the production of IFN-γ by TH1 CD4+ T-lymphocytes, induces the proliferation of thymocytes and mature T-lymphocytes but blocks the IL-2 induced proliferation of peripheral T-cells as well as the production of IL-2 dependent LAK cells. On B-cells, IL-4 has a growth factor activity mediated via the production of soluble CD-23, and a differentiation activity leading to the production of IgE, IgM and IgG1. On monocytes, IL-4 induces an increased number of histocompatibility class II antigens and CD-23 receptors but inhibits the expression of IgG receptors. IL-4 blocks the production of IL-1, IL-6, TNF-α, PGE2, G-CSF and stimulates the production of M-CSF and G-CSF by the monocytes. IL-4 has also an action on eosinophiles by increasing the expression of CD-23 and inhibiting the expression of IgG receptors.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Horizontal microtiter plate shaker capable of 700 rpm ± 100 rpm
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

Procedural Notes

1. When not in use, kit components should be refrigerated.
2. All reagents and samples should be warmed to room temperature before use.
3. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
4. Samples should be collected in pyrogen/endotoxin-free tubes.
5. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
6. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
7. It is recommended that all standards, controls and samples be run in duplicate.
8. Other controls which contain azide will interfere with the enzymatic reaction and cannot be used. Serum or heparin plasma pools as well as stimulated cell culture supernatants can be collected and frozen immediately in aliquot to serve as controls. Repeated freezing and thawing are not permitted.
9. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
13. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
15. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing

- **Incomplete washing will adversely affect the test outcome**. All washing must be performed with the *Wash Buffer Concentrate (200X)* provided.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted *Wash Buffer*. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *Wash Buffer*, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the washing instructions carefully.

Preparation of Samples and Reagents

Sample Preparation

The IL-4 kit may be used to measure IL-4 in serum. Isolation and culture of peripheral blood mononuclear cells may be realized by usual methods. However, one should avoid an unintentional stimulation of the cells by the procedure. The use of pyrogen-free reagents and adequate controls are mandatory.

Sampling conditions can affect values measured in serum, therefore, strict precautions have to be taken during sampling to avoid impurities contained in sampling materials that would stimulate IL-4 production by blood cells and thus falsely increase plasma IL-4 values.

Serum must be removed as soon as possible from the clot of red cells after clotting and centrifugation, and kept at 4°C.

Collection tubes must be pyrogen-free.

Storage: Serum samples must be kept at -20°C for maximum 2 months, and for longer storage (maximum one year) at - 70°C.

Standards, Controls, and Specimen Diluent

Reconstitute the lyophilized Standards, Controls, and Specimen Diluent to the volume specified on the vial label with distilled water: Standard 0 in 5mL distilled water; the other Standards and controls in 1mL distilled water. Allow them to remain undisturbed until completely dissolved, then mix well by gentle inversion. See vial label for exact concentration.

The reconstituted Standards, Controls, and Specimen Diluent are stable for 4 days at 2°C to 8°C. Aliquots held for longer periods of time should be frozen at -20°C (maximum 2 months). Avoid freeze-thaw cycles.

Wash Buffer

Dilute 2 mL of Washing Solution Concentrate in 400 mL distilled water or all the contents of the Wash Solution Concentrate vial in 2000 mL distilled water (use a magnetic stirrer).

The Wash Solution Concentrate is stable at room temperature until expiration date. In order to avoid washerhead obstructions, it is recommended to prepare a fresh diluted Wash Solution each day.

Chromogen Solution

Pipette 0.2 mL of the Concentrated Chromogen (TMB) into one of the vials of Substrate Buffer (H_2O_2 in acetate/citrate buffer). Extemporaneous preparation is necessary. Use only at room temperature. Chromogenic Solution is stable for a maximum of 15 min. Avoid direct exposure to sunlight

The freshly prepared Chromogen Solution is stable for a maximum of 15 min. at room temperature and must be discarded afterwards.

Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Pipette 100 µL of Incubation Buffer into each well.
3. Pipette 100 µL of each Standard, Control, or Sample into the appropriate wells.
4. Pipette 50 µL of anti-IL-4 Conjugate into all the wells.
5. Incubate for **2 hours at room temperature** on a horizontal shaker set at 700 rpm ± 100 rpm.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
7. Pipette 200 µL of freshly prepared Chromogen Solution into each well within 15 min. following the washing step.
8. Incubate the plate for **30 min. at room temperature** on an horizontal shaker set at 700 ± 100 rpm, avoiding direct sunlight. ***Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceeds the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well.
9. Pipette 50 µL of Stop Solution into each well. The solution in the wells should change from blue to yellow.
10. Read absorbance at 450 nm and 490 nm (reference filter: 630 or 650 nm) within 2 hours after adding the *Stop Solution*.
11. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
12. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be further diluted with Standard 0 and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

Typical Data (Example)

The following data were obtained for the various standards over the range of 0 to 1696 pg/mL IL-4.

Standard IL-4 pg/mL	Optical Density (450 nm)
1696	3.631
552	1.709
156	0.571
51	0.207
14.5	0.089
0	0.036

Performance Characteristics

Analytical Sensitivity

The minimum detectable dose of Hu IL-4 is estimated to be 1.2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Precision

1. Intra-Assay Precision

Samples of known Hu IL-4 concentration were assayed in replicates of 20 to determine precision within an assay.

	Sample 1	Sample 2
Mean (pg/mL)	141	536
SD	5.4	19.8
%CV	3.8	3.7

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 20 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2
Mean (pg/mL)	118.7	381.5
SD	5.4	21.2
%CV	4.5	5.6

SD = Standard Deviation
CV = Coefficient of Variation

Linearity of Dilution

Sample	Dilution	Theor.conc. (pg/mL)	Meas. conc. (pg/mL)
Serum	1/1	-	1266.4
	1/2	633.2	531.5
	1/4	316.6	253.1
	1/8	158.3	122.2
	1/16	79.1	65.3

Samples were diluted with Specimen diluent.

Recovery

Sample	Added IL-4 (pg/mL)	Recovered IL-4 (pg/mL)	Recovery (%)
Serum	0	0	
	80.7	85	105
	342	381.3	111
	780	877.3	112

Antigenic Specificity

No significant cross-reaction was observed in presence of 50 ng of IL-1α, IL-1, IL-2, IL-3, IL-6, IL-7, IL-8, IL-10, TNF-β, IFN-α, TGF-β, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, RANTES, PF-4, βTG, GRO, IP-10 and SCF. This IL-4 assay is specific for human natural and recombinant IL-4.

Expected Values

At the present stage of our studies, only preliminary results can be provided and we thus recommend that each laboratory establishes its own normal values. For guidance, the results of 40 serum samples from apparently healthy persons with low CRP levels, ranged between 0 and 38.7 pg/mL. 29 samples obtained values below the detection limit of the test (<1.2 pg/mL).

High Hook Dose Effect

A sample spiked with IL-4 up to 0.5 µg/mL gives a response higher than that obtained for the last standard point.

Limitations of the Procedure

Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native IL-4 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Appendix

Troubleshooting Guide

Elevated background	*Cause:* Insufficient washing and/or draining of wells after washing. Solution containing HRP-Conjugate can elevate the background if residual is left in the well. *Solution:* Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid. *Cause:* Contamination of substrate solution with metal ions or oxidizing reagents. *Solution:* Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil. *Cause:* Contamination of pipette, dispensing reservoir or substrate solution with HRP Conjugate. *Solution:* Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen. *Cause:* Incubation time is too long or incubation temperature is too high. *Solution:* Reduce incubation time and/or temperature.
Elevated sample/ standard ODs	*Cause:* Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly. *Solution*: Follow the protocol instructions regarding the dilution of the standard. *Cause*: Incubation times extended. *Solution:* Follow incubation times outlined in protocol. *Cause:* Incubations carried out at 37°C when RT is dictated. *Solution:* Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.
Poor standard curve	*Cause:* Improper preparation of standard stock solution. *Solution:* Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample. *Cause:* Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted. *Solution:* NEVER substitute any components from another kit. *Cause*: Errors in pipetting the standard or subsequent steps. *Solution*: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no color develops	*Cause:* Reagents not at RT (25 ± 2°C) at start of assay. *Solution:* Allow ALL reagents to warm to RT prior to commencing assay. *Cause:* Incorrect storage of components, e.g., not stored at 2 to 8°C. *Solution:* Store all components exactly as directed in protocol and on labels. *Cause:* TMB solution lost activity. *Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. *Solution 2:* Avoid contact of the TMB solution with items containing metal ions. *Cause:* Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized. *Solution:* Please contact Technical Support for advice when using alternative sample types. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.
Poor Precision	*Cause:* Errors in pipetting the standards, samples or subsequent steps. *Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip. *Cause:* Repetitive use of tips for several samples or different reagents. *Solution:* Use fresh tips for each sample or reagent transfer. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

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2. Miossec, P. et al., (1990). Low levels of interleukin-4 and high levels of transforming growth factor ß in rheumatoid synovitis. *Arth. Rheum*., 33:1180-1186.
3. Yokota, T. et al., (1988). Molecular biology of IL-4 and IL-5 genes and biology of their products that stimulate B-cells, T-cells and hemopoietic cells. *Immunol. Rev*., 102:137-187.

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Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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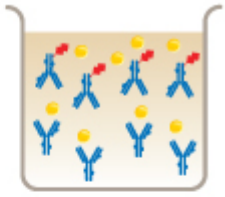
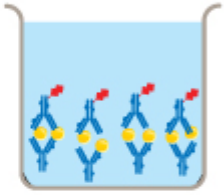
Human IL-4 Assay Summary

Add 100 µL Incubation Buffer
Add 100 µL standards/controls/samples
Add 50 µL anti-IL-4 conjugate
Incubate 2 hr at RT on shaker

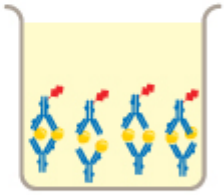
aspirate and wash 4x



Incubate 200 µL of Chromogen
for 30 minutes at RT on shaker



Add 50 µL Stop Solution
Read at 450 nm



Total time: 2 hr 30 minutes