

Revised 2 Dec. 2009 (Vers. 2.0)

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Immunoenzymetric assay for the in vitro quantitative measurement of human interleukin-4 (IL-4) in serum.

2 CLINICAL BACKGROUND

2.1 Biological activities

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 the 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. By its pleiotropic activity, IL-4 is a key cytokine in the cytokine network that shows anti-inflammatory properties and is probably involved in mechanisms of allergy.

3 PRINCIPLES OF THE METHOD

The IL-4-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IL-4. Calibrators and samples react with the capture monoclonal antibody (MAb 1) coated on 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 MAb 1 – human IL-4 – MAb 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the IL-4 concentration.

A calibration curve is plotted and IL-4 concentration in samples is determined by interpolation from the calibration curve. The use of the ELISA reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in a high sensitivity in the low range and in an extended calibration range.

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4 REAGENTS PROVIDED

| | Reagents | 96 tests Kit | Color Code | Reconstitution |
|-----------------------|--|---------------------|------------|--|
| | Microtiterplate with 96 anti IL-4 (monoclonal antibodies) coated wells | 96 wells | blue | Ready for use |
| Ab HRP | HRP Conjugate: HRP labelled anti-IL-4 (monoclonal antibodies) in TRIS-Maleate buffer with bovine serum albumin and thymol | 1 vial 6 ml | red | Ready for use |
| CAL 0 | Zero Calibrator: human serum with benzamidin and thymol | 1 vial lyophil. | yellow | Add 5 ml distilled water |
| CAL N | Calibrator N = 1 to 5 (see exact values on vial labels) in human serum with bezamidin and thymol | 5 vials lyophil. | yellow | Add 1 ml distilled water |
| DIL SPE | Specimen Diluent: human serum with benzamidin and thymol | 2 vial lyophil. | black | Add distilled water (see on the label for the exact volume) |
| INC BUF | Incubation Buffer: Phosphate buffer with bovine serum albumin and thymol | 1 vial 11 ml | black | Ready for use |
| WASH SOLN CONC | Wash Solution Concentrate (Tris-HCl) | 1 vial 10 ml | brown | Dilute 200 x with distilled water (use a magnetic stirrer). |
| CONTROL N | Control - N = 1 or 2 in human serum with thymol | 2 vials lyophil. | silver | Add 1 ml distilled water |
| CHROM TMB CONC | TMB Substrate Concentrate Chromogen TMB (Tetramethylbenzidine) in Dimethylformamide | 1 vial 1 ml | green | Dilute 0.2 ml into 1 vial of substrate buffer |
| SUB BUF | Substrate buffer: H ₂ O ₂ in acetate / citrate buffer | 3 vials 21 ml | white | Ready for use |
| STOP SOLN | Stop Solution: H ₂ SO ₄ , 1.8 N | 1 vial 6 ml | black | Ready for use |

Note: 1. Use Specimen Diluent for sample dilutions.

2. 1 pg of the calibrator preparation is equivalent to 10 mU of the NIBSC 1st IS 88/656

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5 SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. High quality distilled water
2. Pipettes for delivery of: 50 µl, 100 µl, 200 µl, 1 ml and 10 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Horizontal microtiterplate shaker capable of 700 rpm \pm 100 rpm
6. Washer for Microtiter plates
7. Microtiter plate reader capable of reading at 450 nm, 490 nm and 650 nm (in case of polychromatic reading) or capable of reading at 450 nm and 650 nm (bichromatic reading)
8. Optional equipment: The ELISA-AID™ necessary to read the plate according to polychromatic reading (see paragraph 10.1.) can be purchased from Robert Maciels Associates, Inc. Mass. 0.2174 USA.

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6 REAGENT PREPARATION

A. Calibrators:

Reconstitute the zero calibrator with 5 ml distilled water and the other calibrators with 1 ml distilled water.

B. Controls:

Reconstitute the controls with 1 ml distilled water.

C. Specimen diluent:

Reconstitute Specimen diluent to the volume specified on the vial label with distilled water

D. Working Wash solution:

Prepare an adequate volume of Working Wash solution by adding 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize.

Discard unused Working Wash solution at the end of the day.

E. Revelation Solution:

pipette 0.2 ml of the chromogen TMB into one of the vials of substrate buffer (H₂O₂ in acetate/citrate buffer).

Extemporaneous preparation is recommended.

7 STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, calibrators, controls and Specimen diluent are stable for 4 days at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 2 months. Avoid successive freeze thaw cycles.
- The concentrated Wash Solution is stable at room temperature until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the conjugate is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- The freshly prepared revelation solution is stable, before use, for maximum 15 minutes at room temperature and must be discarded afterwards.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

8 SPECIMEN COLLECTION AND PREPARATION

- Serum must be removed as soon as possible from the clot of red cells after clotting and centrifugation, and kept at 4°C. If the samples are not used immediately, they must be kept at -20°C for maximum 2 months, and at -70°C for longer storage (maximum one year).
- Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at room temperature. It is recommended to vortex the samples before use.
- Sampling conditions can affect values, 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 serum IL-4 values.

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- Collection tubes must be pyrogen-free.

9 PROCEDURE

9.1 Handling notes

Do not use the kit or components beyond expiry date.

Do not mix materials from different kit lots.

Bring all the reagents to room temperature prior to use.

Thoroughly mix all reagents and samples by gentle agitation or swirling.

Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.

Use a clean plastic container to prepare the Wash Solution.

In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.

For the dispensing of the Revelation Solution and the Stop Solution avoid pipettes with metal parts.

High precision pipettes or automated pipetting equipment will improve the precision.

Respect the incubation times.

To avoid drift, the time between pipetting of the first calibrator and the last sample must be limited to the time mentioned in section 12.5 (Time delay).

Prepare a calibration curve for each run, do not use data from previous runs.

The Revelation Solution should be colourless. If a blue colour develops within a few minutes after preparation, this indicates that the reagent is unusable, and must be discarded.

Dispense the Revelation Solution within 15 minutes following the washing of the microtiterplate.

During incubation with Revelation Solution, avoid direct sunlight on the microtiterplate.

9.2 Procedure

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the strips into the holding frame.
3. Pipette 100 µl of Incubation Buffer into all the wells
4. Pipette 100 µl of each Calibrator, Control and Sample into the appropriate wells.
5. Pipette 50 µl of anti-IL-4-HRP conjugate into all the wells.
6. Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm \pm 100 rpm.
7. Aspirate the liquid from each well.
8. Wash the plate 3 times by:
 - Dispensing 0.4 ml of Wash Solution into each well
 - Aspirating the content of each well

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9. Pipette 200 µl of the freshly prepared revelation solution into each well within 15 minutes following the washing step.
10. Incubate the microtiter plate for 30 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
11. Pipette 50 µl of Stop solution into each well.
12. Read the absorbencies at 450 nm and 490 nm (reference filter 630 nm or 650 nm) within 3 hours and calculate the results as described in section 10.

10 CALCULATION OF RESULTS

10.1 Polychromatic Reading:

1. In this case, the ELISA-AID™ software will do the data processing.
2. The plate is first read at 450 nm against a reference filter set at 650 nm (or 630 nm).
3. A second reading is performed at 490 nm against the same reference filter.
4. The ELISA-AID™ Software will drive the reader automatically and will integrate both readings into a polychromatic model. This technique can generate OD's up to 10.
5. The principle of polychromatic data processing is as follows:

$X_i = \text{OD at 450 nm}$

$Y_i = \text{OD at 490 nm}$

Using a calibrator unweighted linear regression, the parameters A & B are calculated : $Y = A \cdot X + B$

If $X_i < 3$ OD units, then X calculated = X_i

If $X_i > 3$ OD units, then X calculated = $(Y_i - B) / A$

A 4-parameter logistic curve fitting is used to build up the calibration curve.

The IL-4 concentration in samples is determined by interpolation on the calibration curve.

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10.2 Bichromatic Reading

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. On semi-logarithmic or linear graph paper plot the OD values (ordinate) for each calibrator against the corresponding concentration of IL-4 (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

11 TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

| IL-4-ELISA | | OD units Polychromatic model |
|------------|------------|------------------------------|
| Calibrator | 0 pg/ml | 0.036 |
| | 14.5 pg/ml | 0.089 |
| | 51 pg/ml | 0.207 |
| | 156 pg/ml | 0.571 |
| | 552 pg/ml | 1.709 |
| | 1696 pg/ml | 3.631 |

12 PERFORMANCE AND LIMITATIONS

12.1 Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two calibrator deviations above the average OD at zero binding, was 1.2 pg/ml.

12.2 Specificity

No significant cross-reaction was observed in presence of 50 ng of IL-1 α , IL-1 β , IL-2, IL-3, IL-6, IL-7, IL-8, IL-10, TNF- β , IFN- α , TGF- β , GM-CSF, MIP-1 α , MIP-1 β , MCP-1, G-CSF, RANTES, PF4, GRO, IP-10 and SCF.

This IL-4 assay is specific for human natural and recombinant IL-4.

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12.3 Precision

| INTRA ASSAY | | | | INTER ASSAY | | | |
|-------------|----|--------------------|-----------|-------------|----|--------------------|-----------|
| Serum | N | <X> ± SD (U/ml) | CV (%) | Serum | N | <X> ± SD (U/ml) | CV (%) |
| A | 20 | 141.1 ± 5.4 | 3.8 | A | 20 | 118.7 ± 5.4 | 4.5 |
| B | 20 | 536.3 ± 19.8 | 3.7 | B | 20 | 381.5 ± 21.2 | 5.6 |

SD: Calibrator Deviation; CV: Coefficient of variation

12.4 Accuracy

RECOVERY TEST

| Sample | Added IL-4 (pg/ml) | Recovered IL-4 (pg/ml) | Recovery (%) |
|--------|-----------------------|---------------------------|-----------------|
| Serum | 0 | 0 | - |
| | 80.7 | 85 | 105 |
| | 342 | 381.3 | 111 |
| | 780.8 | 877.3 | 112 |
| | 0 | 0 | - |
| | 80.7 | 92 | 114 |
| | 342 | 401.4 | 117 |
| | 780.8 | 876 | 112 |

DILUTION TEST

| Sample | Dilution | Theoretical Concent. (pg/ml) | Measured Concent. (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.

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12.5 Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrators have been added to the coated wells.

| Sample | 0 min | 10 min | 20 min | 30 min | 40 min |
|--------|-------|--------|--------|--------|--------|
| 1 | 36.5 | 37.1 | 34.0 | 33.7 | 35.2 |
| 2 | 78.12 | 73.3 | 67.6 | 68.3 | 69.2 |
| 3 | 351.1 | 346.0 | 345.1 | 328.3 | 338.7 |
| 4 | 985.0 | 871.9 | 899.6 | 894.6 | 908.0 |

12.6 Hook effect

A sample spiked with IL-4 up to 0.5 µg/ml gives higher OD's than the last calibrator point.

13 INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls that contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.
- It is recommended that controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

14 REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of 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).

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15 PRECAUTIONS AND WARNINGS

Safety

For in vitro use only.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with all reagents, Stop Solution contains H₂SO₄, the chromogen contains TMB in Dimethylformamide, Substrate buffer contains H₂O₂. In case of contact, wash thoroughly with water.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

16 BIBLIOGRAPHY

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2. MIOSSEC P. et al., (1990).
Low levels of interleukin-4 and high levels of transforming growth factor β 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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17 SUMMARY OF THE PROTOCOL

| | CALIBRATORS (µl) | SAMPLE(S) CONTROLS (µl) |
|---|----------------------------|---------------------------------------|
| Incubation Buffer | 100 | 100 |
| Calibrators (0-5) | 100 | - |
| Samples, Controls | - | 100 |
| Anti-IL-4 -HRP conjugate | 50 | 50 |
| <p>Incubate for 2 hours at room temperature with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash Solution and aspirate.</p> | | |
| Revelation Solution | 200 | 200 |
| <p>Incubate for 30 min at room temperature with continuous shaking at 700 rpm.</p> | | |
| Stop Solution | 50 | 50 |
| <p>Read on a microtiter plate reader and record the absorbance of each well at 450 nm (and 490 nm) versus 630 nm (or 650 nm)</p> | | |











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








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SYMBOLS USED WITH DRG ASSAYS

| Symbol | English | Deutsch | Français | Español | Italiano |
|---|------------------------------------|------------------------------|--|---------------------------------------|-------------------------------------|
|  | Consult instructions for use | Gebrauchsanweisung beachten | Consulter les instructions d'utilisation | Consulte las instrucciones de uso | Consultare le istruzioni per l'uso |
|  | European Conformity | CE-Konformitätskennzeichnung | Conformité aux normes européennes | Conformidad europea | Conformità europea |
|  | In vitro diagnostic device | In-vitro-Diagnostikum | Usage Diagnostic in vitro | Para uso Diagnóstico in vitro | Per uso Diagnostica in vitro |
|  | For research use only | Nur für Forschungszwecke | Seulement dans le cadre de recherches | Sólo para uso en investigación | Solo a scopo di ricerca |
|  | Catalogue number | Katalog-Nr. | Numéro de catalogue | Número de catálogo | Numero di Catalogo |
|  | Lot. No. / Batch code | Chargen-Nr. | Numéro de lot | Número de lote | Numero di lotto |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze | Contenu suffisant pour "n" tests | Contenido suficiente para <n> ensayos | Contenuto sufficiente per "n" saggi |
|  | Storage Temperature | Lagerungstemperatur | Température de conservation | Temperatura de conservación | Temperatura di conservazione |
|  | Expiration Date | Mindesthaltbarkeits-datum | Date limite d'utilisation | Fecha de caducidad | Data di scadenza |
|  | Legal Manufacturer | Hersteller | Fabricant | Fabricante | Fabbricante |
| Distributed by | Distributor | Vertreiber | Distributeur | Distribuidor | Distributore |
| Content | Content | Inhalt | Conditionnement | Contenido | Contenuto |
| Volume/No. | Volume / No. | Volumen/Anzahl | Volume/Quantité | Volumen/Número | Volume/Quantità |

| Symbol | Portugues | Dansk | Svenska | Ελληνικά |
|---|--------------------------------------|--|---|---------------------------------------|
|  | Consulte as instruções de utilização | Se brugsanvisning | Se bruksanvisningen | Εγχειρίδιο χρήστη |
|  | Conformidade com as normas europeias | Europaeisk overensstemmelse | Europeisk överensstämmelse | Ευρωπαϊκή Συμμόρφωση |
|  | Diagnóstico in vitro | In vitro diagnostik | Diagnostik in vitro | in vitro διαγνωστικό |
|  | | | | |
|  | Catálogo n.º | Katalognummer | Katalog nummer | Αριθμός καταλόγου |
|  | No do lote | Lot nummer | Batch-nummer | Αριθμός Παρτίδος |
|  | | Indeholder tilstrækkeligt til "n" test | Innehåller tillräckligt till "n" tester | Περιεχόμενο επαρκές για «n» εξετάσεις |
|  | Temperatura de conservação | Opbevarings-temperatur | Förvaringstemperatur | Θερμοκρασία αποθήκευσης |
|  | Prazo de validade | Udløbsdato | Bäst före datum | Ημερομηνία λήξης |
|  | Fabricante | Producent | Tillverkare | Κατασκευαστής |
| Distributed by | | | | |
| Content | Conteúdo | Indhold | Innehåll | Περιεχόμενο |
| Volume/No. | Volume/Número | Volumen/antal | Volym/antal | Όγκος/αριθ.. |