



User's Manual

Human IL 12 p40 ELISA Kit

REF DEIA2021

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Immunoenzymetric assay for measurement of human interleukin-12 (IL-12) in serum.

General Description

Human interleukin-12 (IL-12) is a 75 kDa lymphokine produced mainly by monocytes, macrophages, B-lymphocytes and dendritic cells. IL-12 shows an unusual heterodimeric structure composed of one 40 kDa (p40) and of one 35 kDa (p35) subunits linked together by disulfide bonds. p35 subunit is distantly related to IL-6 and G-CSF while p40 shows homology to the extracellular domain of the α chain of the IL-6 receptor. This suggests that IL-12 may have evolved from a cytokine/soluble receptor complex.

p40 is secreted in large excess over the biologically active heterodimer. p40 is involved in receptor binding but p35 is necessary

for signal transduction. Monomers and mainly homodimers of p40 show antagonist activity to IL-12.

In vivo, IL-12 appears to play a major role in auto-immune disease, in the resistance to bacterial and parasitic infections, in

antiviral responses including HIV, in the promotion of antitumor immunity. IL-12 has been shown to be a powerful adjuvant in

vaccination.

Principles of Testing

The IL-12+p40-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IL-12. 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-12 – 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-12 concentration. A calibration curve is plotted and IL-12 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.

Reagents And Materials Provided

The Human IL 12 p40 ELISA Kit supplies sufficient materials for 96 determinations.

Materials Required But Not Supplied

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 ± 100 rpm
6. Washer for Microtiterplates
7. Microtiterplate 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 can be purchased from Robert Maciels Associates, Inc. Mass. 0.2174 USA.

Storage

1. 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.
2. Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
3. 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.
4. The concentrated Wash Solution is stable at room temperature until expiration date.
5. Freshly prepared Working Wash solution should be used on the same day.
6. After its first use, the conjugate is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
7. The freshly prepared revelation solution is stable, before use, for maximum 15 minutes at room temperature and must be discarded afterwards.
8. Alterations in physical appearance of kit reagents may indicate instability or deterioration.

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 4C. 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-12 production by blood cells and thus falsely increase serum IL-12 values.

Collection tubes must be pyrogen-free.

Reagent Preparation

A. Calibrators:

Reconstitute the 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.

Assay Procedure

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored¹. 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. Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
6. Aspirate the liquid from each well.
7. Wash the plate 3 times.
8. Dispensing 0.4 ml of Wash Solution into each well
9. Aspirating the content of each well.
10. Pipette 100 µl Specimen Diluent and 50 µl of anti-IL-12-HRP conjugate into all the wells.
11. Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
12. Aspirate the liquid from each well.
13. Wash the plate 3 times.
14. Dispensing 0.4 ml of Wash Solution into each well.
15. Aspirating the content of each well.

16. Pipette 200 µl of the freshly prepared Revelation Solution into each well within 15 minutes following the washing step.
17. Incubate the microtiterplate for 30 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
18. Pipette 50 µl of Stop Solution into each well.
19. Read the absorbencies at 450 nm and 490 nm (reference filter 630 nm or 650 nm) within 3 hours and calculate the results.

Quality Control

1. 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.
2. 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.
3. Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
4. 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.
5. It is good practise to check visually the curve fit selected by the computer.

Calculation

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 standard 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-12 concentration in samples is determined by interpolation on the calibration curve.

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-12 (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.

Typical Standard Curve

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

Reference Values

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

For guidance, the results of 26 serum samples from apparently healthy persons with low CRP levels, ranged between 24 and 369 pg/ml with a mean value of 136 pg/ml.

Sensitivity

The minimum detectable dose of IL 12 is < 2.0 pg/ml.

Specificity

No significant cross-reaction was observed in presence of 150 ng of IL-1 α , IL-1 β , IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL10, IL-15, TNF- α , TNF- β , IFN- γ , IP-10, ScF, GRO α , OSM, LIF, MCP-1, MCP-3, MIP-1 α , MIP-1 β , RANTES, NAP-2, PDGF, GCSF, GMCSF, GP-130 & sIL-6R.

Precautions

1. 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.
2. 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.

3. 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.
4. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.