GE Healthcare

Amersham Interleukin-12 [(h)IL-12], (p40 and p70), Human, Biotrak ELISA System

96 wells

Product Booklet

Code: RPN2765

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1. Legal

First published 1998.

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2. Handling

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at 2–8°C.

2.3. Expiry

The expiry date is stated on the package and will be at least 4 weeks from the date of despatch

3. Components of the assay system

This pack contains the following assay components, sufficient material for 96 wells. All reagents are stored refrigerated at 2–8°C. Refer to the expiry date on the kit box.

(h)IL-12 microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human IL-12.

(h)IL-12 standard - 2 vials of prediluted natural IL-12, lyophilized.

FITC antibody reagent - prediluted antibody against (h)IL-12 conjugated to FITC, with preservative, 12 ml.

HRP-antibody conjugate - pre-diluted antibody to FITC, conjugated to HRP, with preservative, 12 ml.

IL-12 standard diluent - with preservative, 12 ml.

Pre-mixed TMB substrate reagent - with preservative, 12–13 ml.

Wash buffer concentrate - 30-fold concentrated solution, 50 ml.

Stop solution - <1% sulfuric acid, 13–15 ml

Plate covers - 4 adhesive strips

4. Description

The Biotrak™ human interleukin-12 ELISA system from GE Healthcare provides a simple, specific and precise quantitative determination of (h)IL-12 in cell culture supernatants, plasma and serum.

The assay is based on a solid phase ELISA, which utilizes an antibody for (h)IL-12 bound to the wells of a microplate together with a fluorescein labeled antibody to (h)IL-12 and horseradish peroxidase conjugated to anti-FITC antibody (figure 1).

(h)IL-12 can be measured in the range 25.6–1000 pg/ml (1.25–50 pg/well) in less than 4 hours using the protocol provided with the kit. Each pack contains sufficient material for 96 wells. If one standard curve is constructed, 42 unknowns can be measured in duplicate.

- High sensitivity <5 pg/ml (0.25 pg/well)
- Same day protocol
- Pre-coated plate
- Specific for (h)IL-12 (p40 and p70)

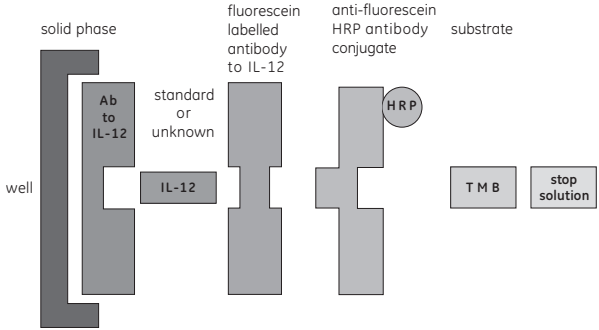

Figure 1: Human Interleukin-12 ELISA assay design

5. Critical parameters

- Allow samples and all reagents to reach room temperature prior to performing the assay. Do not use water baths to thaw samples.
- Mix samples and all reagents thoroughly before use.
- Avoid excessive foaming of reagents. Also avoid exposure of reagents to excessive heat or light during storage and incubation.
- Avoid handling the tops of the wells both before and after filling.
- Standards and samples should be assayed in duplicate.
- Run a separate standard curve for each assay.
- The total dispensing time for each plate should not exceed 20 minutes.
- Use only coated wells from the same reagent batch for each assay. Also do not mix reagents from different kit lots.
- For sample and conjugate incubations a humidified incubator may be used to prevent evaporation loss due to incomplete plate sealing.
- It is important that the wells are washed thoroughly and uniformly. If using an automatic washer check operation of head before starting. If washing by hand ensure that all wells are completely filled at each wash.
- A small amount of precipitate may be present in some vials. It will not affect assay performance and should be ignored.
- Use a new adhesive plate cover for each incubation step in the ELISA.
- New pipette tips should be used for each standard and sample. GE Healthcare recommends the use of multiple tips or multi-shot dispensing pipettes for the addition of other reagents to the wells.

6. Additional materials and equipment required

The following materials and equipment are required:

- Pipettes or pipetting equipment with disposable tips (50 µl, 100 µl, and 1.0 ml)
- Disposable polypropylene test tubes - do not use polystyrene, polycarbonate or glass
- Measuring cylinder 2 l
- Distilled or deionized water
- Plate reader capable of reading at 450 nm
- Wash bottle or automatic plate washer

Optional equipment

Assays may be performed with commercially available microplate washers to aid convenience and assay throughput.

7. Specimen collection and sample preparation

Sample collection

The assay is provided for the quantitative determination of human IL-12 levels in serum, EDTA and citrated plasma and cell culture supernatant. Use of heparinized plasma will result in values approximately 30% lower than those of serum.

Prior to assay, slowly thaw frozen sera, plasma or culture supernatants at 2–8°C and gently mix by hand. Do not thaw samples in a 37°C water bath.

Serum, plasma, culture fluids

Clarify serum and plasma samples containing a visible precipitate prior to assay. Do not use grossly hemolyzed or lipemic specimens. Store at -70°C. Avoid more than one freeze/thaw cycle. It is recommended that the samples be kept at 2–8°C during the handling process.

Dilution of test samples

If it is suspected that the (h)IL-12 concentration of a sample exceeds the highest point of the standard curve, prepare one or more five-fold dilutions of the test sample. Mix thoroughly between dilutions and before assaying.

8. ELISA procedure

8.1. Reagent preparation

All reagents are supplied ready to use, except for those described below.

Wash buffer concentrate

Add contents of the wash buffer concentrate vial to 1450 ml of deionized water and mix well.

8.2. Preparation of working standards

1. Two vials of lyophilized standard are provided with this kit. Reconstitute and use one vial per partial plate.
2. Prepare standards shortly before use. Use within one hour of reconstitution.

   Do not store reconstituted standards
3. When running **culture supernatant samples**, reconstitute standard in distilled or deionized water. Reconstitution volume is stated on the standard vial label. The standard will take approximately 1 minute to dissolve. Mix by gently inverting the vial. Use your culture medium to prepare the dilutions of the standard curve, proceed to step 5 below for further instructions. If running a partial plate, refer to step 1 above.
4. If running serum or plasma samples, reconstitute standard with distilled or deionized water. Reconstitution volume is stated on the standard vial label. The standard will take approximately 1 minute to dissolve. Mix by gently inverting the vial. Use the standard diluent provided to prepare the dilutions of the standard curve. If running a partial plate, refer to step 1 above.

5. Label 6 tubes, one for each standard curve point: 1000 pg/ml, 400 pg/ml, 160 pg/ml, 64 pg/ml, 25.6 pg/ml and 0 pg/ml. Then prepare 1:2.5 serial dilutions for the standard curve as follows:
6. Pipette 240 µl of appropriate diluent (see steps 3 and 4 above) into each tube.
7. Pipette 160 µl of the reconstituted standard into the first tube labeled 1000 pg/ml and mix.
8. Pipette 160 µl of this dilution into the second tube labeled 400 pg/ml and mix.
9. Repeat serial dilutions three more times. These concentrations, 1000 pg/ml, 400 pg/ml, 160 pg/ml, 64 pg/ml, 25.6 pg/ml and 0 pg/ml are your standard curve points.

8.3. Assay protocol

This ELISA provides the flexibility to run two partial plates on separate occasions. Decide the number of strips you wish to run, leaving the strips to be used in the frame. Remove the unnecessary strips and store them in the foil pouch with the desiccant provided at 2–8°C, making sure the foil pouch is sealed tightly.

When adding the TMB substrate reagent, pour out from the bottle only the amount needed to run the first half plate. Do not combine left over substrate with that reserved for the second half of the plate. Care must be taken to ensure that the remaining TMB substrate reagent is not contaminated. **If the substrate reagent is bright blue prior to use, it has been contaminated. DO NOT USE.**

1. Prepare assay reagents and working standards as described in the previous sections.
2. Set up the microplate with sufficient wells to enable the running of all standards and samples as required (see figure 2).

3. Remove excess microplate strips from the frame and store in the resealable foil bag.
4. Add 50 µl of standard, sample or diluent (zero standard) to each well that is to be used.
5. Add 100 µl of FITC labeled antibody reagent.
6. Cover with adhesive strip provided and incubate for 2 hours at room temperature (20–25°C).
7. Remove adhesive strip and aspirate or decant each well and wash, repeating the process twice for a total of three washes. Wash vigorously by filling each well with wash buffer using a wash bottle, pipette or manifold dispenser. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towelling.
8. Add 100 µl of HRP-antibody conjugate to all wells. Cover with a new adhesive strip and incubate for 30 minutes at room temperature (20–25°C).
9. Repeat the aspiration/wash step as in step 7.
10. Add 100 µl of TMB substrate solution into each well and incubate for 30 minutes at room temperature (20–25°C) without shaking. If the substrate reagent is bright blue prior to use, do not use. **THE PLATE SHOULD BE DEVELOPED IN THE DARK.**

    Do not cover the plate with aluminium foil or an adhesive strip.
11. Add 100 µl of stop solution to each well.
12. Determine the optical density of each well within 30 minutes, using a spectrophotometer set to 450 nm.

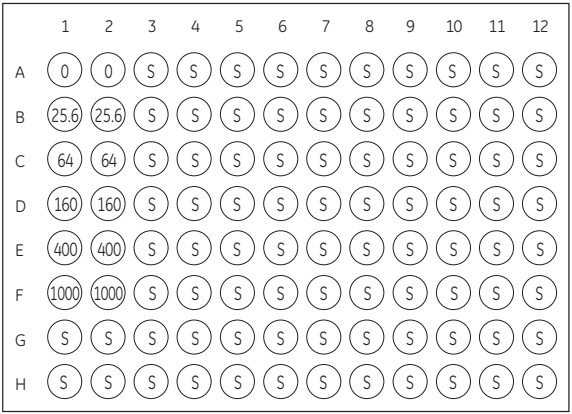

Figure 2. Recommended positioning of standards (25.6–1000 pg/ml) and samples (S).

Table 1. Assay protocol (all volumes are in microlitres)

	Zero standard	Standards	Samples
Standard	–	50	–
Sample diluent	50	–	–
Sample	–	–	50
FITC antibody reagent	100	100	100
Cover plate, incubate at room temperature for 2 hours			
Aspirate/decant and wash vigorously all wells three times with wash buffer			
HRP antibody conjugate	100	100	100
Cover plate, incubate for 30 minutes at room temperature			
Aspirate/decant and wash vigorously all wells three times with wash buffer			
Substrate	100	100	100
Incubate at room temperature without shaking for 30 minutes in the dark			
Stop solution	100	100	100
Determine optical density at 450 nm within 30 minutes			

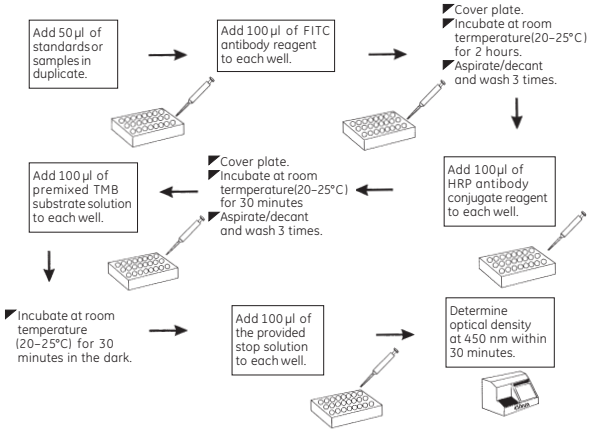

Figure 3. Summary of assay protocol

9. Data processing

9.1. Calculation of results

Typical assay data is shown in table 2.

Average the duplicate readings for each standard and sample and subtract the zero standard optical density.

Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized using a log/log plot and regression analysis can be applied to the log transformation.

Figure 4 shows such a plot of the data from table 2. The standard curve is provided for illustration only. A standard curve should be generated for each set of samples to be assayed. This allows for the measurements of 42 unknowns in duplicate.

9.2. Typical assay data

The following data (table 2) were obtained for a standard curve using the protocol provided.

Table 2. Typical assay data

Tube	Optical density	Zero standard subtracted
Zero standard	0.088	–
25.6 pg/ml standard	0.123	0.035
64 pg/ml standard	0.183	0.095
160 pg/ml standard	0.359	0.271
400 pg/ml standard	0.981	0.893
1000 pg/ml standard	2.106	2.018

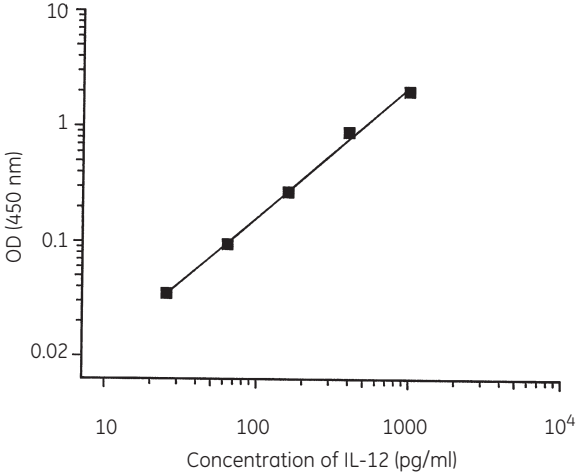

Figure 4. Typical standard curve

10. Additional information

10.1. Specificity

This ELISA is specific for the measurement of natural and recombinant human IL-12. It does not crossreact with human; IL-1α, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-11, IL-13, IL-15, TNFα, TNFβ, IFNγ, IFNα, GM-CSF, mouse IL-12 p40 and mouse IL-12 p70.

10.2. Calibration

The standard consists of a natural mixture of p40 and p70 from the supernatant of Thp-1 cells stimulated with lipopolysaccharide. It is calibrated against human recombinant p70.

10.3. Reproducibility

Within-assay precision

The within-assay coefficient of variation of the ELISA has been determined to be <10%.

Between-assay precision

The between-assay coefficient of variation of the ELISA has been determined to be <10%.

10.4. Sensitivity

The minimum detectable dose of (h)IL-12 was determined to be 5 pg/ml (0.25 pg/well), by adding two standard deviations to the optical density of zero and calculating the corresponding concentration from the standard curve.

10.5. Normal levels

IL-12 levels in normal individuals were determined to be mainly within the range 50–400 pg/ml.

10.6. Recovery

IL-12 from lipopolysaccharide (LPS) stimulated Thp-1 cells was added to serum.

The recovery was as follows:-

Sample type	Mean recovery (n=8)	Range
Serum	86%	77 to 95%

Recovery in various sample matrices was determined by spiking IL-12 from LPS stimulated Thp-1 cells into matched serum and plasma samples prepared from 3 individual human donors. Mean recoveries were compared to that from serum.

Sample type	Mean recovery (n=3)	Range
Serum	100%	–
EDTA plasma	98%	91–102%
Citrate plasma	96%	91–102%
Heparin plasma	71%	66–76%

10.7. Troubleshooting guide

Low optical densities

1. Check the color of the substrate in the wells. If it is blue add acid to terminate the reaction and develop the correct yellow color prior to reading.
2. Check reader wavelength.
3. Ensure all reagents have been equilibrated to room temperature before use.
4. Check reagents have been correctly reconstituted.
5. Check reagents have been stored under the recommended conditions.
6. Check incubation times.

7. Ensure that the plate is read within 30 minutes of adding the stop reagent.

High optical densities

1. Check reagents have been correctly reconstituted.
2. Check incubation times.

Poor replication

1. Ensure automatic washers are working correctly, or that each well is completely filled and emptied at every wash step when hand washing.
2. Check pipette calibration.
3. Ensure troughs used with multichannel pipettes are dedicated to individual components.
4. If splashing occurs when using multishot pipettes, lubricate the pipette barrel.
5. Ensure that no cross contamination occurs by using a fresh adhesive plate cover at each incubation stage.
6. Ensure that plates have been carefully placed into the plate reader to avoid splashing.

Standard replication is good, but the standards do not fit the curve

Check standard reconstitution and dilution procedure.

High non-specific binding

Ensure automatic washers are working correctly, or that each well is completely filled and emptied at every wash step when hand washing.

Brown 'precipitate' in wells

Under conditions where there are very high levels of enzyme activity in the wells, a brown precipitate will appear on addition of acid. This

will initially yield a high optical density but this will decay rapidly. This is indicative of a dilution error with the antibody-HRP conjugate.

10.8. Background and references

IL-12 was initially discovered by its ability to potentiate natural killer cell activity. It is now known to have multiple effects on different lymphocyte populations. In contrast to all known cytokines, which possess only one type of polypeptide chain, IL-12 is unique in that it is a disulphide-linked heterodimer composed of a heavy chain of 40 kDa (p40) and light chain of 35 kDa (p35) encoded by separate genes. The mRNA encoding p35 is constitutively expressed by a variety of cell types, whereas expression of p40 mRNA tends to be more closely correlated with the production of the p75 heterodimer.

Both p40 and a homodimeric form of p40, termed (p40)2 bind to the mouse IL-12 receptor. However, the p40 forms do not trigger biological activity and in fact specifically inhibit IL-12 mediated responses. p40 is produced in large excess over IL-12 and p40 has been proposed to be a natural IL-12 antagonist.

Recent studies have demonstrated that IL-12 is a key cytokine which orchestrates the immune response to invading microorganisms. Its biological activity has been comprehensively reviewed by Ricardo T. Grazzinelli. In brief, IL-12 is considered to be important in controlling pathogens during the early stages of infection. Macrophages stimulated by microbes release IL-12 which acts upon natural killer (NK) cells to release IFNγ. IFNγ is a potent stimulator of macrophage effector functions against invasive pathogens. IL-12 also plays a key role in biasing T-cell differentiation towards the Th1 phenotype. Murine Th1 lymphocytes exclusively producing IL-2, IFNγ and TNFβ are responsible for specific cell-mediated immunity. In contrast, murine Th2 cells exclusively produce IL-4, IL-5, IL-10 and IL-13 and mediate humoral immunity. Successful resolution of many infectious diseases is dependent upon the appropriate induction of a Th1 or Th2

response. The use of IL-12 or IL-12 antagonists in human disease is likely to be an extremely interesting and valuable scientific research field.

1. TREMBLEAU, S. *et al., Immunology Today,* **16**, pp.383-386, 1995.

2. LAMONT, A.G. and Adorini, L., *Immunology Today,* **17**, pp.214-217, 1996.

3. GAZZINELLI, R.T., *Molecular Medicine Today*, **2**, pp.258-267, 1996.

10.9. Related products

Biotrak range of Easy ELISA systems

Interferon-alpha [(h)IFNα]	RPN5960
Interferon-gamma [(h)IFNγ]	RPN5961
Interleukin 1β [(h)IL-1β]	RPN5971
Interleukin-2 [(h)IL-2]	RPN5965
Interleukin-2 [(m)IL-2]	RPN5966
Interleukin-6 [(h)IL-6]	RPN5968
Interleukin-8/Neutrophil-activating peptide-1-1 [(h)IL-8/NAP-1]	RPN5969
Interleukin-10 [(m)IL-10]	RPN5963
Interleukin-13 [(h)IL-13]	RPN5972
Monocyte chemoattractant protein-1 [(h)MCP-1]	RPN5964
Transforming growth factor beta-1 [(h)TGFβ_1]	RPN5970
Tumor necrosis factor-alpha [(h)TNFα]	RPN5967

Biotrak range of human cytokine and growth factor ELISA systems

Interleukin-1α [(h)IL-1α]	RPN2750
Interleukin-1β [(h)IL-1β]	RPN2751
Interleukin-6 [(h)IL-6]	RPN2754
Interleukin-8 [(h)]IL-8]	RPN2764
Interleukin-10 [(h)IL-10]	RPN2755
Interferon-gamma [(h)IFNγ]	RPN2757
Tumor necrosis factor-alpha [(h)TNFα]	RPN2758
Vascular Endothelial Growth Factor [(h)VEGF]	RPN2779

Biotrak range of high sensitivity human cytokine and growth factor ELISA systems

Interferon-gamma [(h)IFNγ]	RPN2787
Interleukin-6 [(h)IL-6]	RPN2784
Interleukin-10 [(h)IL-10]	RPN2785
Tumor necrosis factor-alpha [(h)TNFα]	RPN2788

Biotrak range of mouse cytokine and growth factor ELISA systems

Interferon-gamma [(m)IFNγ]	RPN2707
Interleukin 1β [(m)IL-1β]	RPN2720
Interleukin-4 [(m)IL-4]	RPN2712
Interleukin-6 [(m)IL-6]	RPN2708
Interleukin-12 [(m)IL-12]	RPN2702
Tumor necrosis factor-alpha [(m)TNFα]	RPN2718

Biotrak range of rat cytokine and growth factor ELISA systems

Cytokine-induced neutrophil chemoattractant [(r)GRO/C1NC-1]	RPN2730
Interleukin-1β [(r)IL-1β]	RPN2743
Interleukin-6 [(r)IL-6]	RPN2742
Monocyte chemoattractant protein-1 [(r)MCP-1]	RPN2740
Tumor necrosis factor-alpha [(r)TNFα]	RPN2744

Cell Proliferation assay system and reagents

Cell Proliferation Biotrak ELISA	RPN250
Cell proliferation kit for immunocytochemistry	RPN20
Monoclonal anti-bromodeoxyuridine	RPN202
Cell Proliferation Labeling Reagent	RPN201

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28950783

RPN2765PL Rev AD 01-2009