GE Healthcare

Amersham High Sensitivity Interferon-Gamma [(h) IFNγ] Human, Biotrak ELISA System

96 wells

Product Booklet

Code: RPN2787

Page finder

1. Legal

Previously published 1995

http//www.gehealthcare.com/lifesciences

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK

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 normally 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)IFNg microplate – 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against (h)IFNγ.

Biotinylated antibody reagent – antibody against (h)IFNγ conjugated to biotin, with 0.1% (w/v) sodium azide. 8 ml.

(h)IFNg standard – 2 vials of recombinant human (h)IFNγ, lyophilized.

Amdex™ amplification reagent – lyophilized.

Standard diluent – with 0.1% (w/v) sodium azide, 12 ml, 2 vials.

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

Pre-mixed TMB substrate reagent – 12-13 ml.

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

Plate covers – 6 adhesive strips.

4. Description

The Biotrak™ high sensitivity human interferon gamma ELISA system from GE Healthcare provides a simple, specific, reliable and precise quantitative determination of (h)IFNγ in cell culture supernatants, plasma, serum and urine.

The assay system is based on a solid phase ELISA, which utilizes an antibody for (h)IFNγ bound to the wells of a microplate together with a biotinylated antibody to (h)IFNγ and Amdex amplification reagent. Although the Biotrak (h)IFNγ immunoassay contains recombinant (h)IFNγ and antibodies raised against recombinant (h)IFNγ, it has been shown to quantitate accurately both natural (h)IFNγ and recombinant (h)IFNγ.

(h)IFNγ can be measured in the range 0.63–20 pg/ml (0.063–2 pg/well) in less than 5 hours using the protocol provided with the kit. Each pack contains sufficient material for 96 wells. If one standard curve is constructed, 41 unknowns can be measured in duplicate.

- High sensitivity – 0.1 pg/ml
- Same day protocol
- Pre-coated plate
- Specific for (h)IFNγ

4.1. Summary of the assay system

This assay employs the quantitative 'sandwich' enzyme immunoassay technique. An antibody specific for (h)IFNγ has been coated on the microplate provided in the kit. Samples are pipetted into the wells and the (h)IFNγ, if present, is bound by the immobilized antibody. A biotinylated antibody reagent is added to the wells and allowed to bind to any IFNγ bound by the immobilized antibody in the first incubation. After washing away any unbound biotinylated antibody an Amdex amplification reagent is added. Any IFNγ which

was bound by both the immobilized and the biotinylated antibody will be bound by the amplification reagent. Following a wash to remove unbound amplification reagent, a substrate solution is added to the wells and color develops in proportion to the amount of (h)IFNγ bound in the initial step.

The Amdex amplification reagent is a high performance conjugate based on a novel chemistry that utilizes a hydrophilic straight chain dextran backbone to which many hundreds of horseradish peroxidase molecules are covalently coupled, together with, on average, ten streptavidin molecules. The result is a multifunctional conjugate with a significantly enhanced activity and with well controlled non-specific binding properties.

In addition to the samples to be tested, a series of wells is prepared using known concentrations of the human IFNγ standard. A curve, plotting the optical density versus the concentration of the standard well, is prepared. By comparing the optical density of the samples to this curve, the concentration of the (h)IFNγ in the unknown samples is then determined.

5. Critical parameters

1. Allow samples and all reagents to reach room temperature prior to performing the assays. Do not use water baths to thaw samples or reagents.

2. Mix samples and all reagents thoroughly before use.

3. Avoid excessive foaming of reagents. Also avoid exposure of reagents to excessive heat or light during storage and incubation.

4. Avoid handling the tops of the wells both before and after filling.

5. Standards and samples should be assayed in duplicate.

6. Run a separate standard curve for each assay.

7. The total dispensing time for each plate should not exceed 20 minutes.

8. Use only coated wells from the same reagent batch for each assay. Also do not mix reagents from different kit lots.

9. It is important that all the wells are washed thoroughly and uniformly. If using an automatic washer, check operation of heads before starting. If washing by hand ensure wells are completely filled at each wash.

10. Timings in this assay are critical and should be adhered to strictly. Failure to do so could alter optical densities significantly.

11. A small amount of precipitate may be present in some vials. It will not affect assay performance and should be ignored.

6. Additional materials and equipment required

The following materials and equipment are required:

- Pipettes or pipetting equipment with disposable tips (40 µl, 100 µl, 400 µl, 500 µl, 1.00 ml and 5.00 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
- Plate shaker

Optional equipment

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

7. Sample preparation

Serum, plasma, urine and cell culture supernatants

Serum, plasma, urine and culture supernatant samples that are to be assayed within 24 hours should be stored at 2–8°C. Specimens to be stored for longer periods of time should be frozen at -70°C to avoid loss of activity. Avoid freezing and thawing samples more than once. Test samples should be assayed in duplicate each time the ELISA is performed, 100 µl of sample per well is required in this assay.

The measurement of cytokines in serum and plasma has been reported to be affected by non-specific matrix effects which may vary between samples from different individuals (1–3). Dilution of such samples in the diluent supplied may help to reduce these interference effects.

Dilution of test samples

If it is suspected that the (h)IFNγ 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.

It remains the investigators responsibility to validate the chosen sample dilution.

8. Assay procedure

8.1. Reagent preparation

Wash buffer concentrate

Any precipitate formed during storage will redissolve upon dilution. Dilute the contents of the bottle to 1500 ml using distilled water. Store at 2–8°C until the expiry date of the kit. Do not use wash buffer if it becomes visibly contaminated on storage.

Amdex amplification reagent

Reconstitute the Amdex reagent in 11 ml of distilled or deionized water approximately 15 minutes before use. Reconstituted reagent may be stored at –15°C to –30°C for up to 1 week.

(h)IFNγ standard

Reconstitute 1 vial of (h)IFNγ standard with distilled or deionized water. Reconstitution volume is shown on the vial label. Mix by gently inverting the vial.

It is important that the diluent selected for reconstitution and dilution of the standard reflects the environment of the samples being measured. Standard diluent will be suitable for serum plasma or urine measurements. If your samples are cell culture supernatants, the culture media will be suitable for preparation of the standard curve.

Testing of RPMI with different lots and concentrations of fetal bovine serum has shown that this ELISA is not adversely affected by culture medium. Therefore when culture supernatants, serum plasma or urine samples are assayed on the same plate, standards prepared in standard diluent may be used.

Biotinylated antibody reagent

Dilute the biotinylated antibody reagent 1:1 with standard diluent prior to use as follows:

Pipette 5.5 ml of standard diluent into a suitable container, add 5.5 ml of biotinylated antibody reagent, mix well before use.

8.2. Preparation of standard curve

Preparation of working stock solution

1. Pipette 400 µl of standard diluent into a polypropylene tube.
2. Add to this tube 100 µl of reconstituted (h)IFNg standard and mix thoroughly.
3. This is the 500 pg/ml stock solution from which the working standards are prepared.

Preparation of working standards

1. Label 6 polypropylene tubes, 0.63, 1.25, 2.5, 5, 10 and 20 pg/ml.
2. Pipette 960 µl of standard diluent into the 20 pg/ml tube.
3. Pipette 500 µl of standard diluent into all remaining tubes.
4. Into the 20 pg/ml tube pipette 40 µl of working stock solution of (h) IFNγ and mix thoroughly.
5. Transfer 500 µl from the 20 pg/ml tube to the 10 pg/ml tube and mix thoroughly.
6. Repeat this doubling dilution successively with the remaining tubes.
7. 100 µl aliquots from each serial dilution will give rise to 6 standard levels of (h)IFNγ ranging from 0.63 to 20 pg/ml.

NOTE: Working standards should be freshly prepared before each assay, and not re-used.

Running partial plates

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.**

8.3. Assay protocol

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 1).

3. Remove excess microplate strips from the frame and store in the resealable foil bag.

4. Pipette 100 µl of standard diluent or cell culture medium (see section on reagent preparation) into NSB wells.

5. Pipette 100 µl of standard into the appropriate wells.

6. Pipette 100 µl of sample into the appropriate wells.

7. Cover the plate with the adhesive strip provided and incubate for 1 hour at room temperature (20–25°C) with continuous shaking.

8. Aspirate or decant each well and wash, repeating the process three times for a total of four washes. Wash thoroughly by completely filling each well with wash buffer using a washbottle, 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.

9. Pipette 100 µl of the biotinylated antibody reagent into all wells. Cover with a new adhesive strip and incubate for 2 hours at room temperature (20–25°C) with continuous shaking.
10. Repeat the aspiration/wash step as in step 8.
11. Pipette 100 µl of Amdex amplification reagent to all wells. Cover with a new adhesive strip and incubate for 30 minutes at room temperature (20-25°C) with continuous shaking.
12. Repeat the aspiration/wash step as in step 8.
13. Pipette 100 µl of TMB substrate solution into all wells, incubate for 1 hour at room temperature (20–25°C) with continuous shaking. If the substrate reagent is bright blue prior to use, do not use. Do not cover the plate with aluminium foil or an adhesive strip.
14. Pipette 100 µl of stop solution into all wells.
15. Determine the optical density of each well within 30 minutes, using a spectrophotometer set to 450 nm.

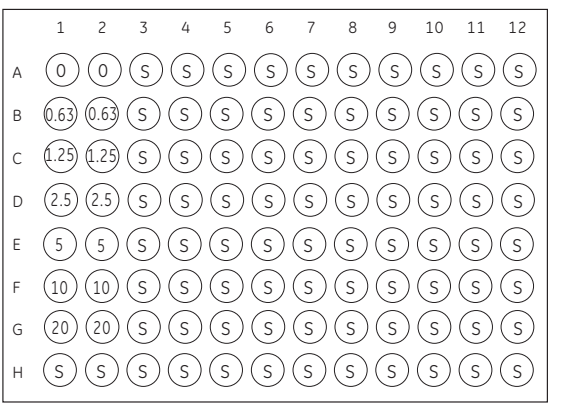

Figure 1. Recommended positioning of standard (0.63–20 pg/ml) and standard wells (S).

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

	Zero standard	Standards	Samples
Standard	–	100	–
Standard diluent or cell culture media*	100	–	–
Sample	–	–	100
Cover plate, incubate at room temperature (20–25°C) on a plate shaker for 1 hour.			
Aspirate/decant and wash thoroughly all wells four times with wash buffer			
Biotinylated antibody reagent	100	100	100
Cover plate, incubate at room temperature (20–25°C) on a plate shaker for 2 hours.			
Aspirate/decant and wash thoroughly all wells four times with wash buffer			
Amdex amplification reagent	100	100	100
Cover plate, incubate at room temperature (20–25°C) on a plate shaker for 30 minutes.			
Aspirate/decant and wash thoroughly all wells four times with wash buffer			
Substrate	100	100	100
Incubate at room temperature (20–25°C) on a plate shaker for 1 hour			
Stop solution	100	100	100
Determine optical density at 450 nm within 30 minutes.			
* Use 100 µl of cell culture media if your standard curve is diluted in cell culture media. See section on reagent preparation.			

9. Data processing

9.1. Calculation of results

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 by using a log/log plot and regression analysis can be applied to the log transformation.

Figure 2 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 measurement of 41 unknowns in duplicate.

9.2. Typical assay data

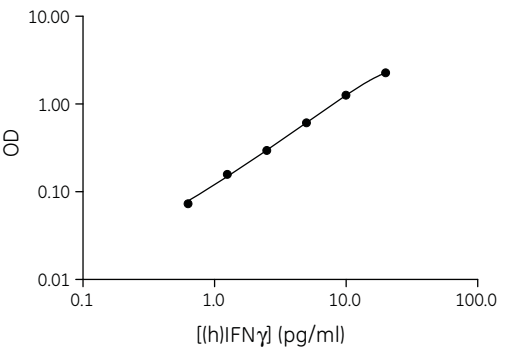

Figure 2. Standard curve

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	0.142	–
0.63 pg/ml standard	0.215	0.073
1.25 pg/ml standard	0.300	0.158
2.5 pg/ml standard	0.438	0.296
5 pg/ml standard	0.753	0.611
10 pg/ml standard	1.401	1.259
20 pg/ml standard	2.408	2.266

When running these high sensitivity assays some variation in OD values may be observed. However control and sample values will not be affected.

10. Additional information

10.1. Specificity

This assay recognizes both natural and recombinant (h)IFNγ . It does not cross react with human IL-1α, IL-1β, IL-2, IL-3, IL-6, IL-7, IL-8, TNFα, IFNγ, GM-CSF or IFNα.

10.2. Calibration

The standards in this ELISA are calibrated to the NIAID reference lot Gxg01-902-535.
1 pg of Biotrak standard = 0.03 NIAID unit.

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%.

Precision profile

A precision profile was generated by preparing replicates of each of the standards and calculating the standard deviation and coefficient of variation at each concentration.

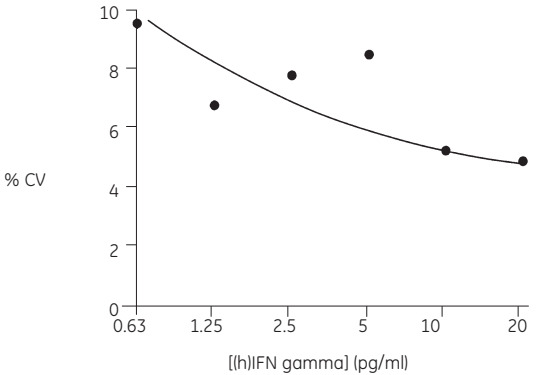

Figure 3. Precision profile

10.4. Sensitivity

The minimum detectable dose of (h)IFNγ was determined to be 0.1 pg/ml (0.01 pg/well), by adding two standard deviations to the optical density value of zero and calculating the corresponding concentration from the standard curve.

10.5. Parallelism

The linearity of dilution was determined by serially diluting six different positive samples. The dilutions were run in the ELISA and 'observed' doses were plotted against 'expected' doses.

10.6. Expected values

Normal levels of (h)IFNγ in serum samples are in the range 0–1.5 pg/ml. Normal levels of (h)IFNγ in plasma samples are in the range 0–2.6 pg/ml. Normal levels of (h)IFNγ in urine samples are in the range 0.5–1.2 pg/ml. Normal average levels of (h)IFNα found in 9 normal urine samples was 4.1 pg/ml.

10.7. Background and references

IFNγ, also known as immune interferon is one of a group of functionally related polypeptide hormones that exhibit potent anti-viral activity both *in vitro* and *in vivo*. IFNγ is unrelated to either IFNα or IFNβ in sequence homology. IFNγ is further distinguished by its expression patterns; IFNγ is restricted to lymphocytes whereas IFNα and IFNβ can be produced by most cell types. In addition to its anti-viral activity, IFNγ activates macrophages to kill both tumour cells and intracellular parasites, enhances NK activity and B cell maturation, proliferation and Ig secretion, induces MHC class I and II antigens, and inhibits osteoclast activation.

Human IFNγ is a 143 amino acid protein with two N-linked glycosylation sites with a molecular weight of 20–25 kDa (determined by SDS PAGE). Production of IFNγ is restricted to activated T cells and NK cells. In both humans and mice the IFNγR is comprised of a ligand binding 90 kDa transmembrane glycoprotein and a less characterized accessory protein necessary for signal transduction. The human and mouse IFNγ binding proteins display 52% homology and bind IFNγ with high affinity ($1\text{-}10 \times 10^{-10}$M) in a species specific manner. IFNγR are present on virtually all cell types, with the exception of erythrocytes, and are abundantly expressed by neural cells. Cell surface receptor densities range from 500–10000 molecules per cell.

The role of IFNγ in several *in vivo* phenomena has clearly been demonstrated. Autoimmune diabetes has been observed in mice transgenic for IFNγ . IFNγ also reduced the morbidity of encephalomyocarditis virus in mice. Mice exposed to Listeria required IFNγ to resolve their infections. IFNγ has also been shown to exhibit anti-tumour effects in a variety of models. IFNγ can be induced by many agents that activate T cells including: mitogenic lectins (PHA and Con A), antigens, IL-1 and IL-2. Agents that inhibit IFNγ synthesis

include: dexamethasone, cyclosporin A and 1α, 25-dihydroxyvitamin D3.

The anti-viral, anti-tumor and anti-parasitic activities displayed by IFNγ suggest that this cytokine may have therapeutic value in a broad spectrum of diseases. In Germany IFNγ is now an approved treatment for rheumatoid arthritis. IFNγ is also being used as an anti-neoplastic agent for a variety of cancers, although initial results have not been encouraging. IFNγ has also demonstrated efficacy in treating viral diseases such as hepatitis B.

1. HENEY, D. and WHICHER, J.T., *Ann. Clin. Biochem.,* **32**, p.358, 1995.

2. de KOSSODO, S. *et al., J. Immunuol. Methods,* **182**, p.107, 1995.

3. WOOD, W.G., *Methods of Immunological Analysis,* MASSEYEFF, R.F. *et al.,* (ED) **Vol. 1**, p.604, 1992.

4. EHLERS, S. *et al., J. Exp. Med.,* **173**, p.25, 1991.

5. KWIATKOWSKI, D. *et al., The Lancet,* **336**, p.1201, 1990.

6. CELADA, A. *et al., Eur. J Immunol.,* **19**, p.1103, 1989.

7. BILLIAU, A., *Immunol. Today,* **9**, p.37, 1988.

8. DOLDI, K. *et al., J. Interferon Research,* **5**, p.55, 1985.

9. ADOLF, G.R., *Oncology,* **42**(1), p.33, 1985.

10. GRAY, P.W. *et al., Nature,* **295**, p.503, 1982.

11. DEVOS, R. *et al., Nucleic Acids Res.,* **10**, p.2487, 1982.

10.8. Related products

Biotrak range of human cytokine ELISA systems

Interleukin-1α [(h)IL-1α]	RPN 2750
Interleukin-1β [(h)IL-1β]	RPN 2751
Interleukin-2 [(h)IL-2]	RPN 2752
Interleukin-6 [(h)IL-6]	RPN 2754
Interleukin-10 [(h)IL-10]	RPN 2755

Granulocyte-macrophage colony stimulating factor [(h)GM-CSF]	RPN 2756
Interferon-gamma [(h)IFNγ]	RPN 2757
Tumor necrosis factor-alpha [(h)TNFα]	RPN 2758
Interferon-alpha [(h)IFNα]	RPN 2759
Interleukin-8 [(h)IL-8]	RPN 2764
Interleukin-12, p40/70 [(h)IL-12] p40/70	RPN 2765
MCP-1 human	RPN 2769

Biotrak range of high sensitivity human cytokine ELISA systems

Interleukin-1α [(h)IL-1α]	RPN 2780
Interleukin-4 [(h)IL-4]	RPN 2783
Interleukin-6 [(h)IL-6]	RPN 2784
Interleukin-10 [(h)IL-10]	RPN 2785
Tumor necrosis factor-alpha [(h)TNFα]	RPN 2788
Interferon-alpha [(h)IFNα]	RPN 2789

Biotrak range of mouse cytokine ELISA systems

Interleukin-1β [(m)IL-1β]	RPN 2720
Interleukin-4 [(m)IL-4]	RPN 2712
Tumor necrosis factor-alpha {(m()TNFα]	RPN 2718

Range of rat cytokine ELISA systems

Range of unlabelled and radiolabelled growth factors and cytokines

Cell proliferation assay system and reagents

Cell proliferation kit (for immunocytochemical/ immunohistochemical measurement)	RPN 20
Monoclonal anti-bromodeoxyuridine	RPN 202
Cell proliferation labelling reagent	RPN 201

GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30 751 84, Uppsala,
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5 D-79111, Freiburg,
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327,
Piscataway, NJ 08855-1327,
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

For contact information for your local office,
please visit: www.gelifesciences.com/contact

GE Healthcare UK Limited
Amersham Place
Little Chalfont, Buckinghamshire,
HP7 9NA, UK

imagination at work

RPN2787PL2 Rev E 06 2008