GE Healthcare

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

96 wells

Product Booklet

Code: RPN2757

Page finder

1. Legal

http//www.gelifesciences.com

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

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

(h)IFNγ standard - 2 vials of prediluted recombinant (h)IFNγ, lyophilized.

Streptavidin-HRP conjugate - streptavidin conjugated to HRP, with preservative, 75 µl.

Streptavidin-HRP dilution buffer - with preservative, 14 ml.

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

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 - 4 adhesive strips.

4. Description

The Biotrak™ 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 a monoclonal antibody for (h)IFNγ bound to the wells of a microplate together with a biotinylated antibody to (h)IFNγ and streptavidin conjugated to horseradish peroxidase. 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 25–1000 pg/ml (1.25–50 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, 42 unknowns can be measured in duplicate.

- High sensitivity - <2 pg/ml (0.1 pg/well)
- Same day protocol
- Pre-coated plate
- Specific for (h)IFNγ

4.1. Summary of the assay

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 along with biotinylated antibody reagent. If present, the (h)IFNγ is bound by the immobilized antibody and the biotinylated antibody. After washing away any unbound sample proteins and biotinylated antibody, a streptavidin-HRP conjugate is added to the wells. Any (h)IFNγ which was bound by both the

immobilized and the biotinylated antibody during the first incubation will be bound by the streptavidin conjugate. Following a wash to remove unbound conjugate, a substrate solution is added to the wells and color develops in proportion to the amount of (h)IFNγ bound in the initial step.

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 standard curve, the concentration of the (h)IFNγ in the unknown samples is then determined.

5. Additional materials and equipment required

The following materials and equipment are required:

- Pipettes or pipetting equipment with disposable tips (50µl, 100 µl and 1.00 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
- A centrifuge for preparing streptavidin-HRP solution

Optional equipment

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

6. 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 biologically active cytokine. Avoid freezing and thawing samples more than once. Test samples should be assayed in duplicate each time the ELISA is performed, 50 µl of sample per well is required in this way.

Dilution of test samples

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

7. Critical parameters

- Allow samples and all reagents to reach room temperature prior to performing the assays. Do not use water baths to thaw samples or reagents.
- 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 heads 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.
- Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

8. Assay procedure

8.1. Reagent preparation

Wash buffer concentrate
Any precipitate formed during storage will redissolve upon dilution. Dilute 30-fold to prepare 1500 ml of wash buffer. Store at 2–8°C until the expiry date of the kit. Do not use wash buffer if it becomes visibly contaminated on storage.

Streptavidin-HRP solution
Prepare the exact amount of streptavidin-HRP solution no more than 15 minutes prior to use.

The streptavidin-HRP concentrate may require spinning down to force the contents to the bottom of the vial. Add 30 µl of streptavidin-HRP concentrate per 12 ml of streptavidin-HRP dilution buffer in a plastic 15 ml tube and mix gently.

If running partial plates use 2.5 µl of streptavidin-HRP concentrate and 1 ml of streptavidin-HRP dilution buffer per strip being run.

Preparation of working standards
Two vials of lyophilized standards are provided with this kit.
Reconstitute and use one vial per partial plate.
Prepare standards shortly before use. Use within one hour of reconstitution.
Do not store reconstituted standards.
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 the appropriate culture medium to prepare the dilutions of the standard curve.

If **running serum, plasma or urine 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.

8.2. Preparation of standard curve

1. 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:
2. Pipette 240 µl of appropriate diluent (see page 11–12) into each tube.
3. Pipette 160 µl of the reconstituted standard into the first tube, 1000 pg/ml and mix.
4. Pipette 160 µl of this dilution into the second tube labelled 400 pg/ml and mix.
5. 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.

8.3. 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.**

8.4. 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. Add 50 µl of biotinylated antibody reagent to each well that is to be used.
5. Add 50 µl of standard or sample per well. Cover with adhesive strip provided and incubate for 2 hours at room temperature (20 to 25°C).
6. 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.
7. Add 100 µl of pre-diluted streptavidin-HRP conjugate. Cover with a new adhesive strip and incubate for 30 minutes at room temperature (20–25°C).
8. Repeat the aspiration/wash step as in step 6.
9. Add 100 µl of TMB substrate solution into each well, incubate for 30 minutes at room temperature (20–25°C). 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.
10. Add 100 µl of stop solution to each well.
11. Determine the optical density of each well within 30 minutes, using a spectrophotometer set to 450 nm.

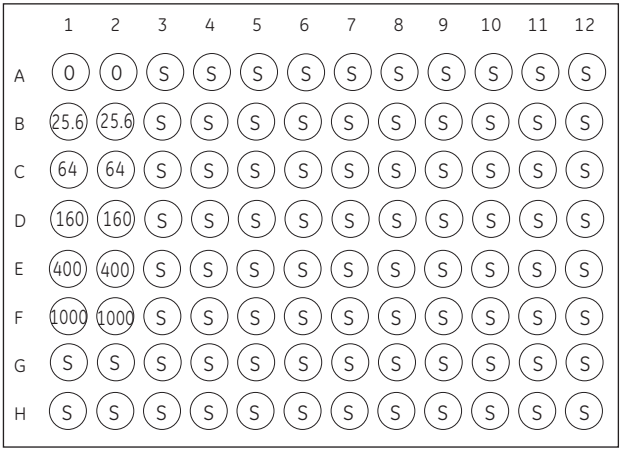
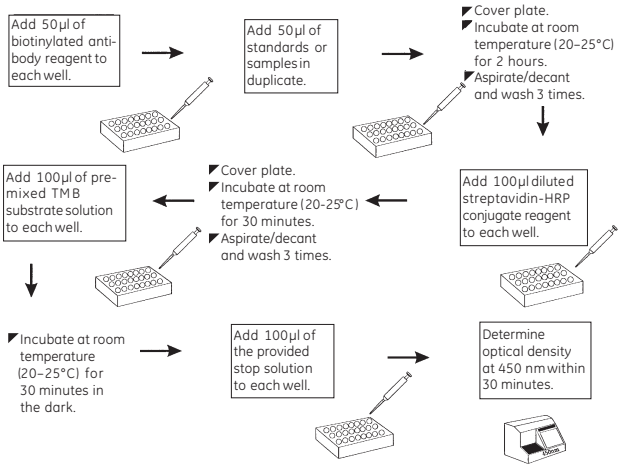


Figure 1. Recommended positioning of standard (0–1000 pg/ml) and sample wells (S).

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

	Zero Standard (B_0)	Standards	Samples
Biotinylated antibody reagent	50	50	50
Standard	–	50	–
Standard diluent or cell culture media	50	–	–
Sample	–	–	50
Cover plate, incubate at room temperature for 2 hours.			
Aspirate/decant and wash vigorously all wells three times with wash buffer.			
Streptavidin-HRP conjugate	100	100	100
Cover plate, incubate at room temperature for 30 minutes.			
Aspirate/decant and wash vigorously all wells three times with wash buffer.			
Substrate	100	100	100
Incubate at room temperature for 30 minutes in the dark.			
Stop solution	100	100	100
Determine optical density at 450 nm within 30 minutes.			

Summary of assay protocol



9. Data processing

9.1. Calculation of results

Average the duplicate readings for each standard, control 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 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 42 unknowns in duplicate.

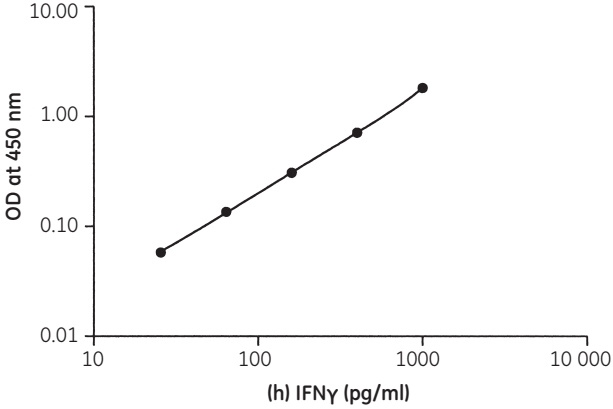


Figure 2. Standard curve

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 subtracted	Zero standard
Zero standard	0.013	–
25.6 pg/ml standard	0.071	0.058
64 pg/ml standard	0.149	0.136
160 pg/ml standard	0.322	0.309
400 pg/ml standard	0.728	0.715
1000 pg/ml standard	1.841	1.828

10. Additional information

10.1. Specificity

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

10.2. Calibration

The standard in this ELISA is calibrated to the NIAID reference lot Gxg01-902-535.

One (1) pg of Biotrak standard = 0.03 NIAID units.

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)IFNγ was determined to be <2 pg/ml (0.1 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. Recovery

Recovery in the ELISA has been determined by spiking 150 pg/ml and 900 pg/ml of recombinant cytokine into samples including individual donors, neat pooled sera, plasma and urine. These were compared to a PBS/BSA solution spiked control. The values overleaf are typical recoveries:

Standard diluent spike	Serum	Plasma	Urine
150 pg/ml	84%	65%	80%
900 pg/ml	106%	95%	105%

10.6. Expected values

The levels found in serum, plasma and urine were:

	Average	Range
Serum (n=35)	0.3 pg/ml	0–1.5 pg/ml
Plasma (n=45)	0.3 pg/ml	0–2.6 pg/ml
Urine (n=5)	0.8 pg/ml	0.5–1.2 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 tumor 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 comprises a ligand binding 90k Da 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 (1-10 x 10-10M) 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 500–10 000 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-tumor 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. EHLERS, S. *et al., J.Exp. Med*,. **173**, p.25, 1991.

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

3. O'GARA, A., *The Lancet, Vol.,* p.1003, 1989.

4. CELANDA, A. *et al., Eur. J. Immunol.,* **19**, p.1103, 1989.

5. BILLIAU, A., *Immunology Today,* **9**, p.37, 1988.

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

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

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

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

10.8. 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
Interleukin-12 p40/70 [(h)IL-12]	RPN2765
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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