DRG® Interferon-γ (96 wells) (EIA-3277 / EIA-3278)

Revised 29 July 2010 rm (Vers. 2.0) **RUO**

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

INTENDED USE

The Human Interferon-gamma (Hu IFN-γ) ELISA is to be used for determination of Hu IFN-γ in human serum, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Hu IFN-γ.

INTRODUCTION

IFN-γ (type 2, immune IFN) is structurally and functionally distinct from type 1 (alpha/beta) interferons and acts on a separate receptor. Only one IFN-γgene has been identified, coding for a 146 amino acid protein that is post-translationally processed into two glycosylated species of 20 and 25 kDa. Native IFN-γ is pH 2-labile, highly basic, and can aggregate to form dimers that are biologically active. IFN-γ is a real lymphokine produced by activated T (and NK) cells. Despite its clear antiviral and cellular growth regulating activities, its immunomodulatory properties are believed to be the most important. IFN-γ is the principal activator of macrophage function, and it also regulates the differentiation of myeloid cells. It plays an important role in the growth and differentiation of cytotoxic (and possibly suppressor) T cells, activates NK cells and acts as a B cell maturation factor. It regulates Ig isotype production and inhibits IgE responses. One of the modes of action of IFN-γ is to induce the expression of membrane proteins, such as class 1 and class 2 MHCantigens and adhesion molecules on various cell types, high affinity Fc receptors for IgG on myelomonocytic cells, etc. Integrated in the cytokine network, IFN-γ interacts with other cytokines, either in a synergistic (e.g., TNF) or antagonistic (e.g., IL-4) fashion. The precise role of IFN-γ in human diseases and therapy is still poorly defined. Clearly, it is involved in the defense against parasites, intercellular pathogens and possibly tumor cells. Its therapeutic administration partially corrects the deficient immune response observed in lepromatous leprosy and the phagocyte defect of patients with X-Linked chronic granulomatous disease. A deficiency in IFNγγ oduction has been related to persistent viral infections (e.g., EBV), and a correlation could be established between the secretion of IFNγ by peripheral blood mononuclear cells during an herpetic infection and the time of a recurrence. A defect in IFN-production has also been recorded in several primary or secondary immunodeficiency states. IFN-γ is seldom detected in the serum of healthy persons. Its production may be demonstrated *in situ* in several inflammatory disorders (e.g., sarcoidosis, rheumatoid arthritis, subacute thyroiditis, polymyositis, and multiple sclerosis). Higher levels of serum IFN-γ are measured during severe parasitic diseases (e.g., *Plasmodium falciparium*); during cytokine (IL-2) therapy; and after the first injections of OKT3.

This kit has been configured for research use only and is not to be used in diagnostic procedures.
Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Hu IFN-γ kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu IFN-γ has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu IFN-γ content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated polyclonal second antibody.

During the first incubation, the Hu IFN-γ antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme,

a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu IFN-γ present in the original specimen.

REAGENTS PROVIDED

Note: Store all reagents at 2 to 8°C.

Reagent	96 Test Kit	192 Test Kit
Hu IFN-γ Standard, recombinant Hu IFN-γ. Contains 0,1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer. Contains 0,1% sodium azide; 25 mL per bottle.	1 bottle	2 bottles
Antibody Coated Wells. 12 x 8 Well Strips.	1 plate	2 plates
Incubation Buffer. Contains 8 mM sodium azide; 12 mL per bottle.	1 bottle	1 bottle
Hu IFN-γ Biotin Conjugate (Biotin-labeled anti-IFN-γ). Contains 0,1% sodium azide; 6 mL per bottle	1 bottle	2 bottles
Streptavidin-HRP (100X). Contains 3,3 mM thymol; 0,125 mL per vial.	1 vial	2 vials
Streptavidin-HRP Diluent. Contains 3,3 mM thymol; 25 mL per bottle.	1 bottle	1 bottle
Wash Buffer Concentrate (25X). 100 mL per bottle.	1 bottle	1 bottle
Stabilized Chromogen, Tetramethylben-zidine (TMB). 25 mL per bottle.	1 bottle	1 bottle
Stop Solution. 25 mL per bottle.	1 bottle	1 bottle
Plate Covers, adhesive strips.	3	6

Disposal Note:
This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)

3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags**. Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples that are >1000 pg/mL should be diluted with Standard Diluent Buffer.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
15. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between Stabilized Chromogen and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Buffer provided. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.
After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under ASSAY METHOD. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
If using an automated washer, the operating instructions for washing equipment should be carefully followed.

REAGENT PREPARATION AND STORAGE

This assay has been calibrated against the WHO reference preparation (87/586) for Hu IFN-γ (NIBSC, Hertfordshire, UK, EN6 3QG). One microgram equals 20,000 International Units.

Reconstitution and Dilution of Hu IFN-γ Standard

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 5000 pg/mL with *Standard Diluent Buffer*.
   Refer to standard vial label for instructions.
   Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution.
   Use standard within 1 hour of reconstitution.
2. Add 0.100 mL of the reconstituted standard to a tube containing
   0.400 mL *Standard Diluent Buffer*. Label as 1000 pg/mL Hu IFN-γ. Mix.
3. Add 0.150 mL of *Standard Diluent Buffer* to each of 6 tubes labeled
   500, 250, 125, 62.5, 31.2 and 15.6 pg/mL Hu IFN-γ.
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

Dilution of Hu IFN-γ Standard

Standard:	Add:	Into:
1000 pg/mL	Prepare as described in Step 2.	
500 pg/mL	0.150 mL of the 1000 pg/mL std.	0.150 mL of the Diluent Buffer
250 pg/mL	0.150 mL of the 500 pg/mL std.	0.150 mL of the Diluent Buffer
125 pg/mL	0.150 mL of the 250 pg/mL std.	0.150 mL of the Diluent Buffer
62.5 pg/mL	0.150 mL of the125 pg/mL std.	0.150 mL of the Diluent Buffer
31.2 pg/mL	0.150 mL of the 62.5 pg/mL std.	0.150 mL of the Diluent Buffer
15.6 pg/mL	0.150 mL of the 31.2 pg/mL std.	0.150 mL of the Diluent Buffer
0 pg/mL	0.150 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay.
Return the *Standard Diluent Buffer* to the refrigerator.

Storage and Final Dilution of Streptavidin-HRP (100X)

Please Note:
The *Streptavidin-HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100X concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP (100X)* to the refrigerator.

Dilution of Wash Buffer

Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.
Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the Procedural Notes/Lab Quality Control section before carrying out the assay.
Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.
Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 50 µL of the Incubation Buffer to all wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 50 µL of the Standard Diluent Buffer to zero wells. Well(s) reserved for chromogen blank should be left empty.
4. Add 50 µL of standards, samples or controls to the appropriate microtiter wells. (See REAGENT PREPARATION AND STORAGE, Section 2.)
5. Pipette 50 µL of biotinylated Hu IFN-γ Biotin Conjugate solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
6. Cover plate with plate cover and incubate for **1 hour and 30 minutes at room temperature.**

7. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
8. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in REAGENT PREPARATION AND STORAGE, Section 3.)
9. Cover plate with the plate cover and incubate for **45 minutes at room temperature.**
10. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
11. Add 100 µL of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue.
12. Incubate for **30 minutes at room temperature and in the dark.** ***Please Note*****: Do not cover the plate with aluminum foil or metalized mylar.** The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the Stop Solution has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
13. Add 100 µL of Stop Solution to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
14. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of Stabilized Chromogen and Stop Solution. Read the plate within 2 hours after adding the Stop Solution.
15. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
16. Read the Hu IFN-γ concentrations for unknown samples and controls from the standard curve plotted in step 15. (Samples producing signals greater than that of the highest standard (1000 pg/mL) should be diluted in Standard Diluent Buffer and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 1000 pg/mL Hu IFN-γ.
Standard Optical Density Hu IFN-γ (pg/mL) (450 nm)

Standard Hu IFN-γ (pg/mL)	Optical Density (450 nm)
0	0.083
	0.070
15.6	0.109
	0.129
31.2	0.150
	0.184
62.5	0.233
	0.248
125	0.428
	0.427
250	0.838
	0.808
500	1.574
	1.570
1000	2.849
	3.040

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 1000 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >1000 pg/mL with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.
The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu IFN-γ in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.
This kit is for research use only.
Not for human therapeutic or diagnostic use.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Hu IFN-γ is <4 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 35 times.

PRECISION

1. Intra-Assay Precision

Samples of known Hu IFN-γ concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	203,4	381,0	898,3
SD	10,5	21	52,8
%CV	5,2	5,5	5,9

SD = Standard Deviation CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 30 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	190.1	398.2	862.9
SD	11.5	23.9	52.8
%CV	6.0	6.0	6.1

SD = Standard Deviation CV = Coefficient of Variation

LINEARITY OF DILUTION

Human serum and cell culture medium containing 10% fetal bovine serum were spiked with Hu IFN-γ and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Serum			Cell Culture		
	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
neat	708	-	-	1045	-	-
1/2	328	354	93	509	523	97
1/4	178	177	101	240	261	92
1/8	91	89	102	115	131	88
1/16	44	44	100	57	65	88
1/32	20	22	91	26	33	79

RECOVERY

The recovery of Hu IFN-γ added to pooled human serum averaged 98% (range: 89% to 106%). The recovery of Hu IFN-γ added to tissue culture medium containing 1% fetal bovine serum averaged 105%, while the recovery of Hu IFN-γ added to tissue culture medium containing 10% fetal bovine serum averaged 111%.

SPECIFICITY

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Hu IFN-γ kit.
Significant cross-reactivity was not observed to any recombinant antigen tested except for rhesus monkey IFN γ, which had 100% cross-reactivity in this assay.
The following substances were tested and found to have no cross-reactivity:
human IL-4, IL-6, IL-10, TNF-α, SCF;
mouse IL-2, IL-4, IL-6, IL-10, IFN-γ;
rat IFN-γ.

HIGH DOSE HOOK EFFECT

A sample spiked with Hu IFN-γ up to 8 ng/mL gives a response higher than that obtained for the last standard point.

REFERENCES / Literature

1. Guerry, D., et al. (1987) J. Immunol. 139:305-312.
2. Landolfo, S., et al. (1985) Science 119:176-179.
3. Murray, H.W., (1988) Ann. Int. Med. 108:595-608.
4. Nathan, C.F., et al. (1986) N. Engl. J. Med. 315:6-12.
5. Snapper, C.M., et al. (1987) Science 236:944-946.
6. Suzuki, Y., et al. (1988) Science 240:516-518.
7. Jelinek, D.F., et al. (1995) J. Immunol. 154:1606-1613.
8. Naldini, A., et al. (1995) Cytokine 7(2):165-170.