DRG® Interferon-β (EIA-2088)

Revised 2 Dec. 2010 rm (Vers. 2.1) **RUO**

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

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

1 INTRODUCTION

This kit used to determine the amount of biologically active human Interferon-β (HuIFN-β) in culture medium and in human plasma or serum.
Although levels HuIFN-β have been determined based on inhibition of the cytopathic effect of viruses on human cells, the bioassay cannot discriminate between the various types of interferons (alpha, beta, and gamma). This HuIFN-β ELlSA kit specific to biologically active HuIFN-β and thus overcomes the lack, of specificity of the bioassay. ELISA is easier and requires no handling of human cells or viruses. Because this assay uses an antibody specific to the biologically active form of HuIFN-β, it correlates well with the bioassay.
The values obtained from the ELlSA can be expressed as international units (IU/mL) since the HuIFN-β standard contained in the kit has been calibrated against the NIH reference for HuIFN-β.'

2 PRINCIPLE

The HuIFN-β ELlSA kit utilizes the one-step sandwich method. Ninety six wells on a microplate are coated with the affinity purified polyclonal antibody to HuIFN-β, in other words the primary antibody is immobilized on the wells.
HuIFN-β in the standard or the sample binds to the antibody coated microwell,. The enzyme (HRP)-labeled anti-HuIFN-β monoclonal antibody also binds to form the antibody-antigen complex where the HuIFN-β is sandwiched between the primary antibody immobilized on the well and the enzyme-labelled secondary antibody. Finally an enzyme reaction system is activated by adding a color developer. The HuIFN-β bound to the solid phase is quantified by the spectrophotometric measurement of the chromogenic substance which develops the color in a reaction catalyzed by enzyme horseradish peroxidase.

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com

3 KIT COMPONENTS AND REAGENT PREPARATION

Allow Dilution buffer and distilled water for use to be under ice cooled condition.

Reagent	Form	Specification	Remarks	Preparation Method	Notes
(1) Antibody coated microplate	Dry wells	96 wells 1 piece, (8 wells x 12 segments)	Wells coated with affinity-purified goat anti-HuIFN-β	Use it following the test procedure.	Allow to come to room temperature prior to use.
(2) Enzyme labelled antibody	Lyoph.	1 vial for 6 mL	Horse radish peroxidase (HRP) labeled mouse monoclonal antibody to HuIFN-β Fab`	**Dissolve** with 6 mL of ice-cooled Dilution Buffer (5).	Place under ice cooled condition prior to preparation.
(3) Human IFN-β Standard	Lyoph.	2 vials for 1 mL	Human fibroblast-derived HuIFN-β. (The titer is described on the label.)	**Reconstitute** lyophilizd standard (3) with 1.0 mL of ice cooled purified water to obtain a working solution with the concentration (IU/mL) described on the label. Dilute the working solution with the ice cooled Dilution Buffer (5) to **make serial dilutions** of the standard at levels of 200 – 100 – 50 – 20 – 10 – 5 and 2.5 IU/mL. Use the Dilution Buffer (5) as standard solution with 0 IU/mL.	Since HuIFN-β is easily denaturated by heat of physical forces, the serial dilutions must be prepared under ice-cooled condition with gentle stirring.
(4) Wash solution	Conc.	1 x 50 mL	Concentrated PBS containing 0.5% Tween 20	**Dilute** entire contents of the concentrated Wash Solution (4) by adding 450 mL of distilled water (Dilute 10 times).	Allow to come to room temperature prior to use.
(5) Dilution Buffer	Liquid	1 x 50 mL	Phosphate buffer containing 0.1% BSA and 0.05% Tween 20	Use following test procedure.	Place under ice cooled condition prior to preparation.
(6) Substrate A	Liquid	1 vial, 12 mL	Sodium acetate citrate buffer containing H_2O_2.	**Prepare** the color developer **immediately prior to use** in the following manner: Mix 10 mL of Substrate A (6) with 0.5 mL of Substrate B (7). When the number of tests to be run is small prepare the necessary amount of 20:1 mixture and store the remainder separately.	Allow to come to room temperature prior to use. Prepare the color developer just before the completion of antigen-antibody reaction.
(7) Substrate B	Liquid	1 vial, 0.6 mL	3,3´,5,5´-tetramethyl-benzidine (TMB) solution		

(8) Reaction stopper	Liquid	1 vial, 12 mL	0.5 mol/L H_2SO_4	Use it following the test procedure.	Allow to come to room temperature prior to use.
(9) Plate cover seal		3 sheets		Use it following the test procedure.	Allow to come to room temperature prior to use.

4 MATERIALS AND EQUIPMENTS NEEDED

1. Microplate reader (spectrophotometer) capable of measuring absorbance up to 2.0 O.D. at 450 nm.
2. Microplate shaker.
3. Microplate washer.
   For manual operation, automatic dispenser, needle dispenser and aspirator or vacuum pump.
4. Micropipettes and dispenser tips
5. Purified water (distilled or deionized)

5 SPECIMEN COLLECTION

Test specimens must be collected in the same manner as routinely used for any clinical laboratory test.
Store them frozen until use.

6 QUALITY CONTROL

Each test run should include the measurement of serial standard dilutions to obtain a calibration curve and to confirm the kit's performance as well. Duplicate measurement of each test specimen are recommended.

7 TEST PROCEDURES

7.1 Reagent Preparation

1. Allow Enzyme labelled antibody and HuIFN-β standard solution to be under ice cooled condition prior to use. Allow other components (Antibody coated microplate, Substrate A and B, Reaction stopper and Plate cover seaI) and Washing solution to come to room temperature prior to use.
   After they come to room temperature, take out the segments of Antibody coated microplate for use, insert other segments into the bag, zip the bag and store it in the refrigerator.
2. Prepare Enzyme-labelled antibody, HuIFN-β standard solution and Washing solution as described in the "KIT COMPONENTS AND REAGENT PREPARATION".
3. Prepare the colour developer solution just before the completion of antigen-antibody reaction.

7.2 Microplate Priming

Fill each well of a Microplate (1) with 400 µL washing solution. Then, remove the solution completely by aspiration. When an aspirator is not available, drain off the solution and dry wells by tapping the microplate upside down onto a paper towel.

7.3 Reaction Procedure

A. Antigen-antibody reaction:

1. Add 50 µL of Enzyme-labelled antibody into each well.
2. Add 100 µL of either specimens or HuIFN-β standard solutions (0 - 200 lU/mL) into each well.
3. Cover the microplate with a plate cover seal and shake it thoroughly with a microplate shaker while incubating for 120 minutes at room temperature (20 °C -30 °C).

B. Washing:

1. After completion of the antigen-antibody reaction, remove the test specimen well by well with an aspirator. If an aspirator is not available turn the microplate over to drain off the test specimen and dry wells by tapping the microplate upside down onto a paper towel.
2. Fill each well of the microplate with 400 µL of Washing solution. Then, remove the fluid completely by aspiration. When an aspirator is not available, drain off the fluid and dry wells by tapping the microplate upside down onto a paper towel.
3. Repeat step B.2 twice more.

C. Enzyme reaction:

1. Add 100 µL of Color developer into each well.
2. Cover the microplate with a plate cover seal and shake it thoroughly with the microplate shaker while incubating for 30 minutes at room temperature (20 °C – 30 °C).
3. Add 100 µL of Reaction stopper (8) to each well in the same order as step C-1.

D. Measurement:

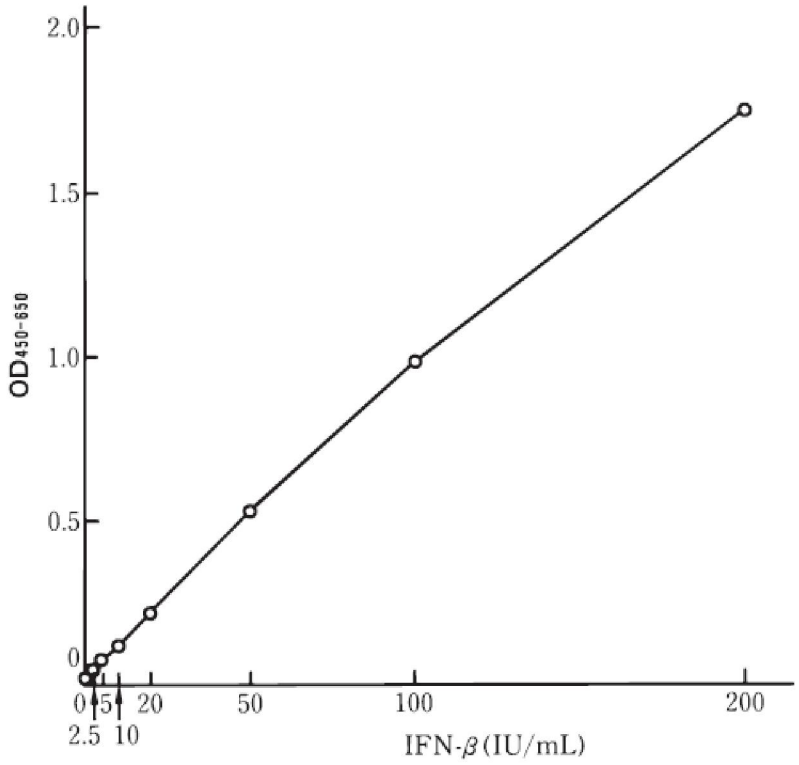
1. Read the absorbance of the reaction mixture in each well at 450 nm against an air blank with a microplate reader. When double wavelength measurement is possible, set the reference wavelength at a range from 590 to 680 nm.
2. Draw a calibration curve of the absorbance at 450 nm versus the concentration of HuIFN-β at each of eight different dilution points.
3. Read out the concentration of HuIFN-β in each specimen from the above calibration curve.

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com

8 SUMMARY OF PROCEDURE

	Standard solution	Samples
Washing	400 µL × once	
Enzyme-labeled antibody Standard solution samples	50 µL 100 µL –	50 µL – 100 µL
Antigen-antibody reaction	120min. at r.t. (with a shaker)	
Washing	400 µL × 3 times	
Color developer	100 µL	100 µL
Enzyme reaction	30min. at r.t. (with a shaker)	
Reaction stopper	100 µL	100 µL
Measurement	450nm (reference 590-680nm)	

Calibration curve (typical)



9 PROCEDURAL NOTES

1. Since the HuIFN-β standard dissolved in distilled water is easily denatured by heat or physical forces, the serial dilutions must be performed under ice-cooled condition with gentle stirring.
2. Specimens should be stored below -20°C. Avoid repeated thawing and freezing.
3. Reagents must be prepared or diluted immediately prior to use and use a new tube must be used for each preparation.
   However, diluted Washing solution can be stored at 2 – 10 °C for 6 month.
   Enzyme-labelled antibody may be stored at 2 – 10 °C for 14 days.
   Reconstituted HuIFN-β standard working solution cannot be stored, and should thus be prepared and diluted immediately prior to use.
4. Although the concentrated Washing solution (4) may produce precipitates during storage, these may be dissolved at the preparation of Washing solution.
5. All specimens and standards should be assayed in duplicate.
6. Since the dispensing procedure influences assay results, make sure it is carried out precisely. To prevent errors caused by contamination among specimens, use a new micropipette tip for every specimen. A new tip should be also used to dispense the Colour developer.
7. When the concentration of HuIFN-β exceeds 200 IU/mL dilute the specimens with Dilution buffer 85).

8. All reactions in the above test procedure should be carried out on a microplate shaker, since the degree of color development is decreased in stationary reactions. Set the shaker speed such that the reaction fluid is fully stirred but does not cause it to splash on the cover seal.
9. The microplate shaker may become hot over longer periods of shaking and raise the temperature of the microplate on it. Avoid this by placing an adiabatic material (e.g. a foam plastic board) between the microplate and the shaker.
10. Measure the absorbance immediately after stopping the reaction.
11. Since reactions may be influenced by various factors, such as time, and temperature and shaking speed, be sure to plot the standard curve for each measurement.
12. Avoid exposure to strong light during the reaction.
13. Since Substrate (b) contains 40% of N,N-Dimethylformamide (ICSC No. 0457, UN No. 2265, EC No. 616-001-00-X), handle the reagent with care.
    Health hazard information: Contact irritant for eyes, skin and mucous membranes. Harmful if inhaled or ingested. Inhalation and ingestion may cause irritation of throat, nausea and headache. It can be absorbed into the body through skin contact and may damage the liver.
    Handling: Wear appropriate personal protective equipment to prevent skin contact. Avoid contact with skin, eyes and clothing. Wash hands and face after handling. Avoid inhalation of gas or mist.
    Storage: Keep container tightly closed, and store away from sunlight. Keep cool at 2-10°C.
    First aid measures: In case of contact with eyes, immediately flush eyes with plenty of water. In case of contact with skin, wash off immediately with soap and plenty of water. If swallowed, take plenty of water to vomit the substance. If irritation persists, seek medical attention.
14. Caution: Substrate (b) is flammable.

10 STORAGE OF REAGENTS

The kit reagents should be stored at 2 °C – 10 °C. DO NOT FREEZE.

11 EXPIRATION DATE

The expiration date is 12 months after manufacturing.

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com