MANUAL

HTRF Human IFNβ Detection Kit

Part # 62HIFNBPEG & 62HIFNBPEH

Test size: 500 tests (62HIFNBPEG), 10,000 tests (62HIFNBPEH) - assay volume: 20 µL

Revision: 05 of September 2023

Store at: ≤-16°C

This product is intended for research purposes only. The product is not intended to be used for therapeutic or diagnostic purposes.

ASSAY PRINCIPLE

Revvity's human IFNβ assay is only intended for the quantitative measurement of IFNβ in supernatant using HTRF® technology. The assay is compatible with human samples, and is highly specific for IFNβ.

IFNβ is detected in a sandwich assay format using 2 different specific antibodies, one labeled with Europium Cryptate (donor) and the second with d2 (acceptor).

The detection principle is based on HTRF® technology. When the labelled antibodies bind to the same antigen, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). The two antibodies bind to the IFNβ present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the IFNβ concentration. (Fig. 1).

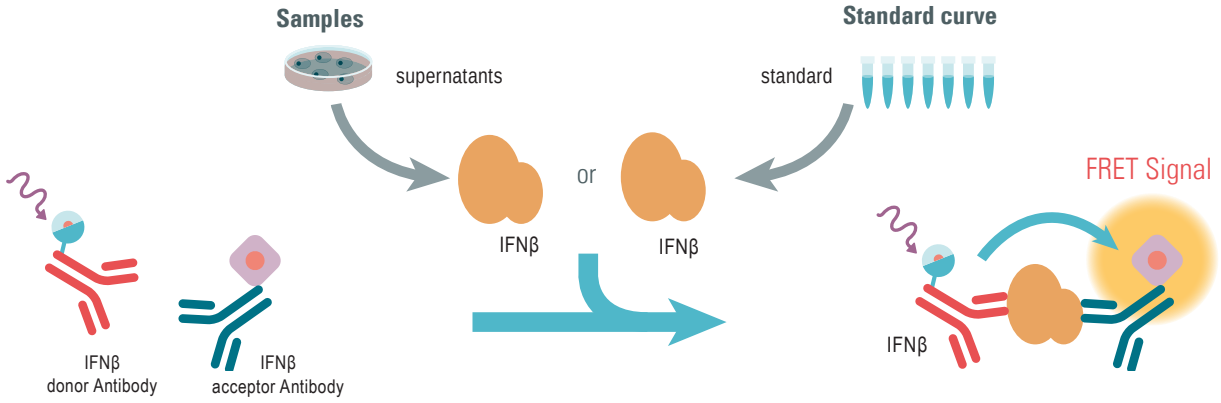
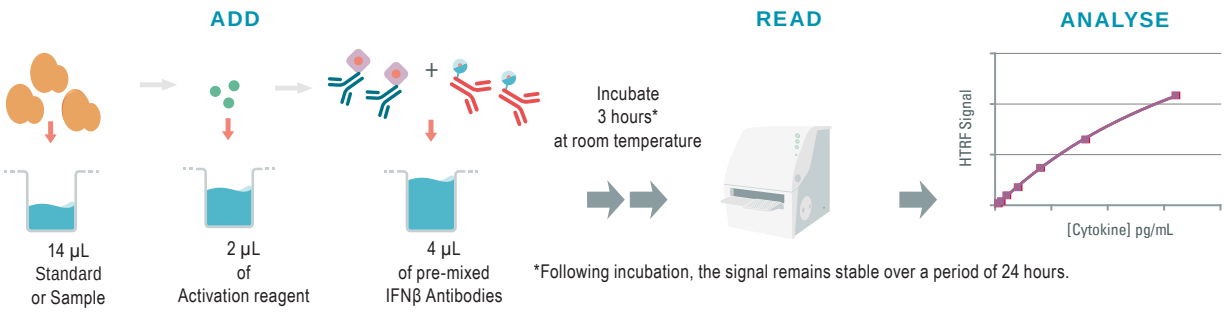


Figure 1: Principle of the HTRF IFNβ sandwich assay.

MANUAL AT A GLANCE



White 384-well low volume plate

For a good performance of the assay, the addition of the Activation Reagent is mandatory.

Make sure to use the set-up for Eu^{3+} Cryptate.
For more information about set-up and compatible HTRF® readers,
please visit our website at: www.revvity.com

MATERIALS:

KIT COMPONENTS	500 TESTS CAT # 62HIFNBPEG	10,000 TESTS CAT # 62HIFNBPEH
IFNβ Standard Lyophilized	2 vials	2 vials
IFNβ Eu Cryptate Antibody Frozen - 20 X	1 vial - 50 µL	1 vial - 1 mL
IFNβ d2 Antibody Frozen - 20 X	1 vial - 50 µL	1 vial - 1 mL
Activation Reagent * ready-to-use	1 vial - 1 mL	1 vial - 20 mL
Diluent ** #5 5X	1 vial 2 mL	1 vial 10 mL
Detection Buffer *** #3 ready-to-use	2 vials 1.5 mL	1 vial 50 mL

* The activation reagent is mandatory and enable epitopes accessibility to HTRF antibodies
** To prepare working standard solutions, culture medium can be an alternative the diluent.
*** The Detection Buffer is used to prepare working solutions of acceptor and donor reagents.

FOR READING, AN HTRF®-CERTIFIED READER IS NEEDED.

For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com

PURCHASE SEPARATELY

96-well or 384-well small volume (SV) detection microplates - For more information about microplate recommendations, please visit our website at: www.revvity.com

STORAGE AND STABILITY

Store the kit at ≤-16°C. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

Once reconstituted, standard stock solution may be frozen, and can be thawed only once..
Once thawed, antibody solutions can be frozen once.
To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at ≤-60°C.

Volume of standard and antibody aliquots should not be under 10 µL.

Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

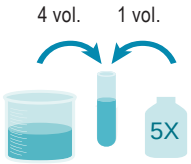
REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature.
- Before use, allow all kit's reagents to warm up at room temperature then
  - homogeneize buffer and diluent with a vortex
  - centrifuge (NEVER vortex) the antibodies to gather all liquid at the bottom of the vial
- It is recommended to filter buffers before use.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.

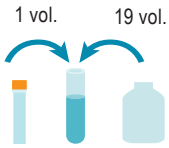
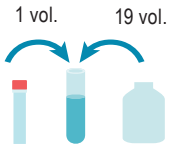
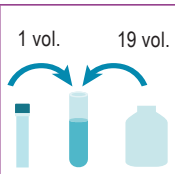
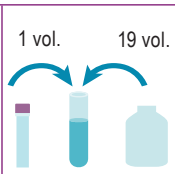
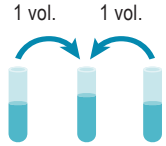
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE DILUENT, STANDARD & ANTIBODY STOCK SOLUTIONS:

500 TESTS		10,000 TESTS
IFNβ Eu Cryptate antibody		
Thaw the IFNβ Eu Cryptate antibody. Centrifuge. This 20 X stock solution can be frozen and stored at ≤-60°C.		Thaw the IFNβ Eu Cryptate antibody. Centrifuge. This 20 X stock solution can be frozen and stored at ≤-60°C.
IFNβ d2 antibody		
Thaw the IFNβ d2 antibody. Centrifuge. This 20 X stock solution can be frozen and stored at ≤-60°C.		Thaw the IFNβ d2 antibody. Centrifuge. This 20 X stock solution can be frozen and stored at ≤-60°C.
IFNβ Standard		
Reconstitute the IFNβ standard with distilled water. Volume of reconstitution is indicated on the vial label. The reconstituted standard solution can be frozen and stored at -60°C or below.		Reconstitute the IFNβ standard with distilled water. Volume of reconstitution is indicated on the vial label. The reconstituted standard solution can be frozen and stored at -60°C or below.
Diluent		
Dilute 5-fold the 5 X diluent #5 with distilled water: Homogenize the 5 X diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water e.g. 1 mL of diluent + 4 mL of distilled water Mix gently after dilution.		Dilute 5-fold the 5 X diluent #5 with distilled water: Homogenize the 5 X diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water e.g. 10 mL of diluent + 40 mL of distilled water Mix gently after dilution.

TO PREPARE WORKING ANTIBODY SOLUTIONS:

Each well requires 4 µL of pre-mixed IFNβ antibodies. Prepare the two antibody solutions in separate vials.

500 TESTS		10,000 TESTS	
IFNβ Eu Cryptate antibody			
Dilute 20-fold the 20 X stock solution (thawed reagent) of IFNβ Eu Cryptate antibody with detection buffer #3: e.g. 10 µL of thawed Eu Cryptate antibody stock solution + 190 µL of detection buffer.			Dilute 20-fold the 20 X stock solution (thawed reagent) of IFNβ Eu Cryptate antibody with detection buffer #3: e.g. 10 µL of thawed Eu Cryptate antibody stock solution + 190 µL of detection buffer).
IFNβ d2 antibody			
Dilute 20-fold the 20 X stock solution (thawed reagent) of IFNβ d2 antibody with detection buffer #3: e.g. 10 µL of thawed d2 antibody stock solution + 190 µL of detection buffer.			Dilute 20-fold the 20 X stock solution (thawed reagent) of IFNβ d2 antibody with detection buffer #3: e.g. 10 µL of thawed d2 antibody stock solution + 190 µL of detection buffer.
Antibody mix			
Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents: e.g. 200 µL of d2 antibody + 200 µL of Eu Cryptate antibody			Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents: e.g. 200 µL of d2 antibody + 200 µL of Eu Cryptate antibody

TO PREPARE WORKING STANDARD SOLUTIONS:

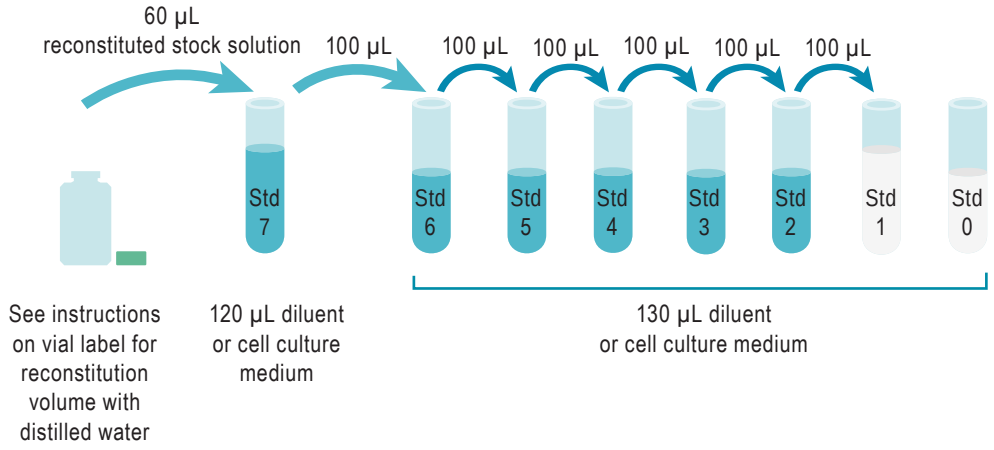
- Each well requires 14 µL of standard.
- Serially dilute the standard stock solution with with diluent #5 or with the cell culture medium used to prepare your samples, supplemented with BSA or 10% FCS.
- Due to the stability of the IFNβ, it is mandatory to prepare the standard curve just before the assay.
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

1. Reconstitute the standard vial with the volume indicated on the vial label using distilled water.

2. Prepare the following dilutions:

• Dilute the reconstituted standard stock solution 3-fold with diluent or with cell culture medium.
In practice: take 60 µL of stock solution and add it to 120 µL of diluent or cell culture medium. Mix gently. This yields the high standard (Std 7: 4,000 pg/ml) for the top of the curve.
•.Use the high standard (Std 7) to prepare the standard curve using serial dilutions as follows:
- Dispense 130 µL of diluent or cell culture medium into each vial from Std 6 to Std 0
- Add 100 µL of standard to 130 µL of diluent or cell culture medium, mix gently and repeat the serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1
This will create 7 standards for the analyte. Std 0 (Negative control) is diluent or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS
Standard Stock solution	Reconstitute the vial following the indications given on the vial label	12 ng/mL
Standard 7	60 µL reconstituted standard stock solution + 120 µL diluent	4,000 pg/ml
Standard 6	100 µL Standard 7 + 130 µL diluent	1,739 pg/ml
Standard 5	100 µL Standard 6 + 130 µL diluent	756 pg/ml
Standard 4	100 µL Standard 5 + 130 µL diluent	329 pg/ml
Standard 3	100 µL Standard 4 + 130 µL diluent	143 pg/ml
Standard 2	100 µL Standard 3 + 130 µL diluent	62 pg/ml
Standard 1	100 µL Standard 2 + 130 µL diluent	27 pg/ml
Standard 0	130 µL diluent	0

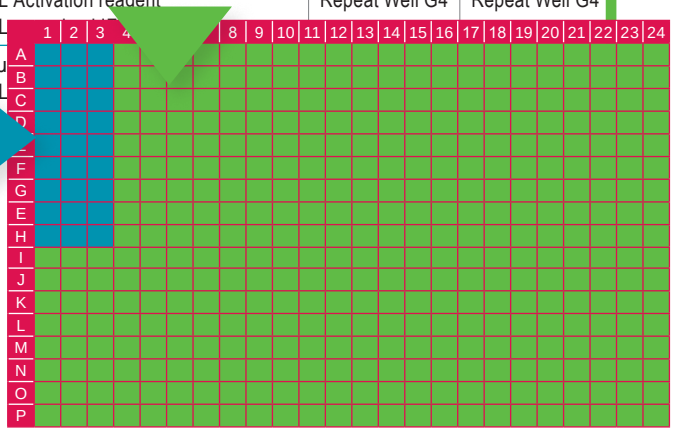
TO PREPARE SAMPLES:

- Each well requires 14 µL of sample.
- Just after their collection, store the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at ≤-60°C. Avoid multiple freeze/thaw cycles.
- All samples with a concentration above the highest standard (Std 7) must be diluted in diluent #5 or in your cell culture medium.

ASSAY MANUAL

	STANDARD (STD 0 - STD 7)	SAMPLES
Step 1	Dispense 14 µL of each IFNβ standard (Std 0 - Std 7) into each standard well.	Dispense 14 µL of each sample into each sample well.
Step 2	Dispense 2 µL of Activation reagent into all wells.	
Step 3	Dispense 4 µL of pre-mixed IFNβ antibodies working solution into all wells.	
Step 4	Seal the plate and incubate 3 hours* at room temperature. *Following incubation, the signal remains stable over a period of 24 hours.	
Step 5	Remove the plate sealer and read on an HTRF® compatible reader.	

	1	2	3	4	5	6
A	14 µL Std 0 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well A1	Repeat Well A1	14 µL Sample 1 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well A4	Repeat Well A4
B	14 µL Std 1 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well B1	Repeat Well B1	14 µL Sample 2 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well B4	Repeat Well B4
C	14 µL Std 2 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well C1	Repeat Well C1	14 µL Sample 3 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well C4	Repeat Well C4
D	14 µL Std 3 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well D1	Repeat Well D1	14 µL Sample ... 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well D4	Repeat Well D4
E	14 µL Std 4 2 µL Activation rReagent 4 µL pre-mixed IFNβ antibodies	Repeat Well E1	Repeat Well E1	14 µL Sample ... 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well E4	Repeat Well E4
F	14 µL Std 5 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well F1	Repeat Well F1	14 µL Sample ... 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well F4	Repeat Well F4
G	14 µL Std 6 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well G1	Repeat Well G1	14 µL Sample ... 2 µL Activation reagent 4 µL	Repeat Well G4	Repeat Well G4
H	14 µL Std 7 2 µL Activation reagent 4 µL pre-mixed IFNβ antibodies	Repeat Well H1	Repeat Well H1	14 µ 2 µL		



DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the delta ratio of the acceptor and donor emission signals for each individual well. The Standard 0 (Negative control) plays the role of an internal assay control.

$$\text{delta Ratio} = \text{Ratio Standard or sample} - \text{Ratio Standard 0}$$

3. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

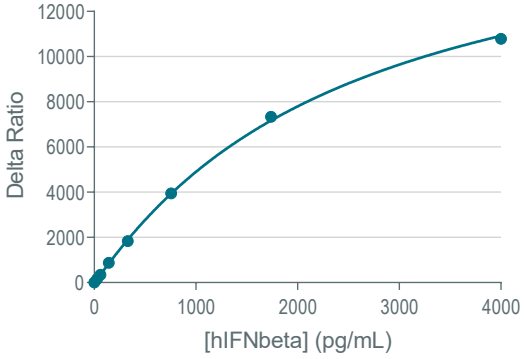
For more information about data reduction, please visit www.revvity.com

RESULTS

This data must not be substituted for the data obtained in the laboratory, and should be considered only as an example. Results may vary from one HTRF® compatible reader to another.

Standard curve fitting with the 4 Parameter Logistic (4PL 1/y2) model. For more information about curve fitting please visit www.revvity.com

		Ratio (1)	delta R (2)	CV% (3)
Standard 0	Negative control	512	0	5%
Standard 1	27 pg/ml	667	155	5%
Standard 2	62 pg/ml	860	348	3%
Standard 3	143 pg/ml	1382	871	3%
Standard 4	329 pg/ml	2348	1836	1%
Standard 5	756 pg/ml	4452	3940	2%
Standard 6	1,739 pg/ml	7842	7330	2%
Standard 7	4,000 pg/ml	11292	10780	1%



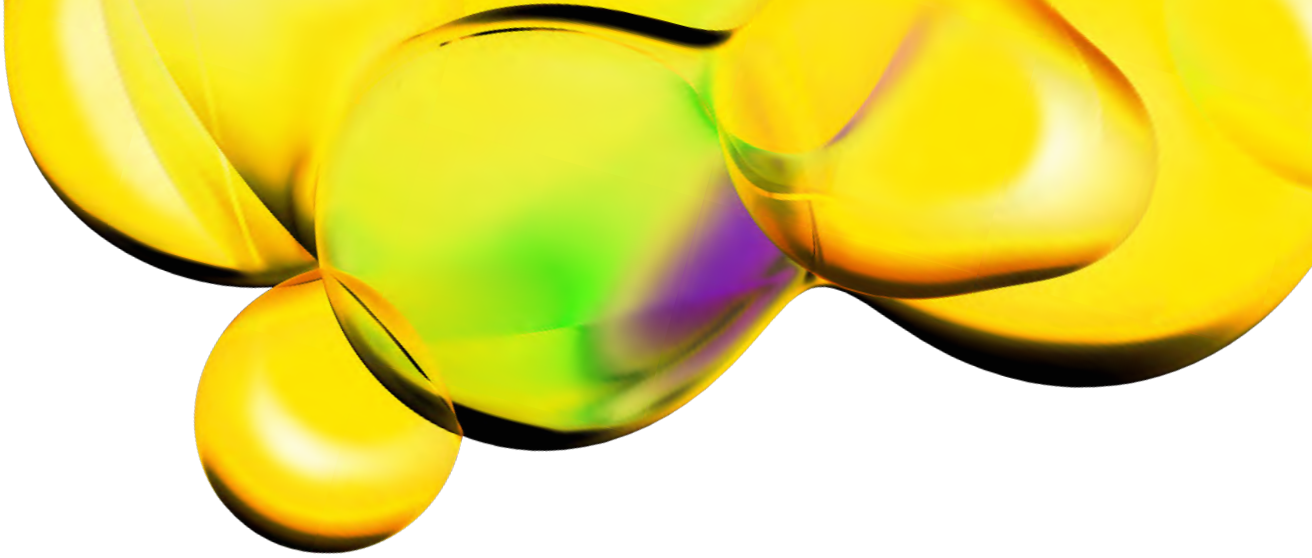
ANALYTICAL ASSAY PERFORMANCE

	Diluent	DMEM	RPMI
Assay range (pg/mL**)	19 pg/mL to 4,000 pg/ml		
Limit of detection (LoD*) = Std 0 mean + 2 SD	7 pg/mL	21 pg/mL	20 pg/mL
Limit of quantification (LoQ*)	19 pg/mL		
Incubation time	3 hours at room temperature		

** NIBSC (00/572) value (IU/ml) = 0.44 x HTRF hIFNβ value (pg/mL)

* The analytical sensitivity was calculated from data obtained with an HTRF compatible reader after 3 hours incubation, this may vary from one HTRF compatible reader to another.

This product contains material of biologic origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception,handling and storage.
The use of the cell line will be done with appropriate safety and handling precautions to minimize health and environmental impact.



Manufactured by **Cisbio** Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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