MANUAL

HTRF Human IFNγ Detection Kit

Part # 62HIFNGPET

Test size: 1 x 96 tests (62HIFNGPET) - assay volume: 20 µL

Revision: #09 of September 2023

Store at: ≤-60°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 IFNγ assay is only intended for the quantitative measurement of IFNγ in supernatant using HTRF® technology. The assay is compatible with human samples, and is highly specific for IFNγ.

IFNγ is detected in a sandwich assay format using 2 different specific antibodies, one labeled with Europium Cryptate (donor) and the second with XL (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 IFNγ present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the IFNγ concentration. (Fig. 1).

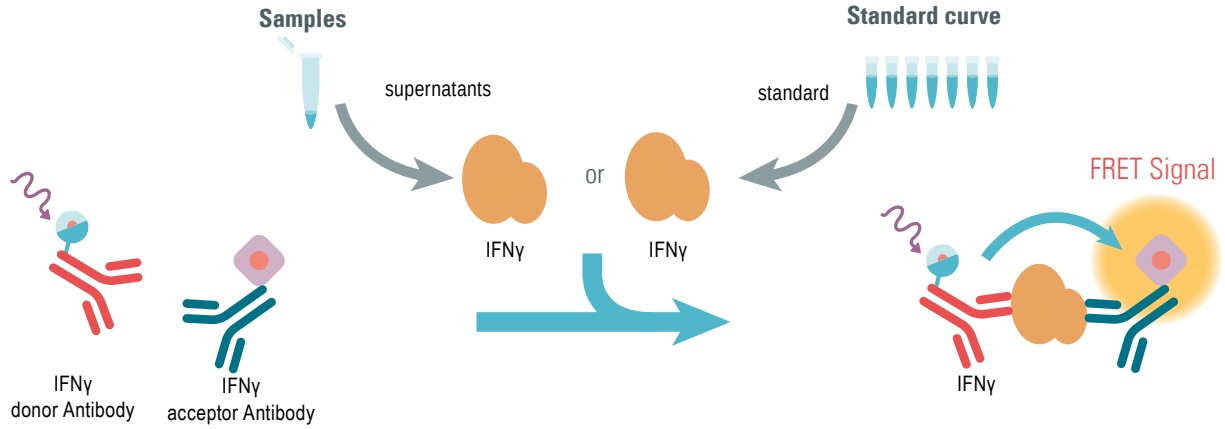
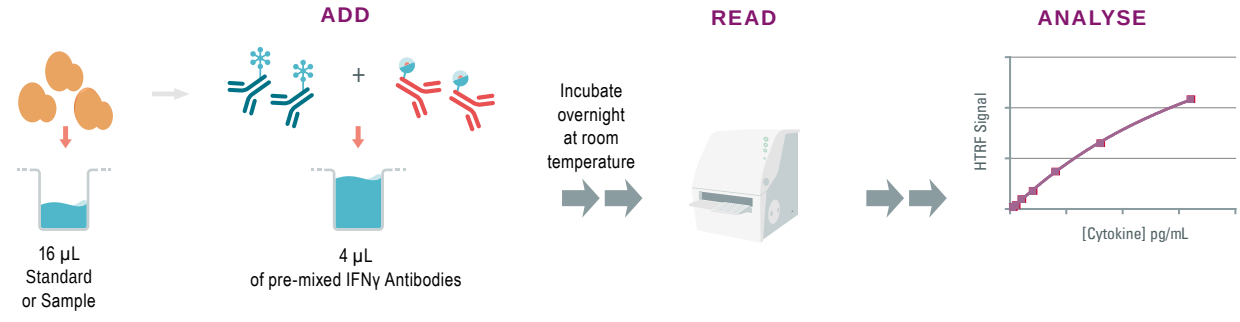


Figure 1: Principle of HTRF IFNγ sandwich assay.

MANUAL AT A GLANCE



White 384-well low volume plate

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	1 X 96 TESTS - CAT # 62HIFNGPET
IFNγ Standard Lyophilized	1 vial
IFNγ Eu Cryptate Antibody Frozen	1 vial - 10 µL
IFNγ-XL Antibody Frozen	1 vial - 10 µL
Diluent* #5 5X	1 vial 2 mL
Detection Buffer** #3 ready-to-use	1 vial - 0.5 mL
Plate	1 plate HTRF 96-well low volume plate

* 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 ≤-60°C. Under proper storage conditions, reagents are stable until the expiry date indicated on the label.

Due to the stability of the IFNγ, it is mandatory to prepare the standard curve just before the assay.
Any remaining reconstituted analyte should be discarded.
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 antibody aliquots should not be under 10 µL.

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

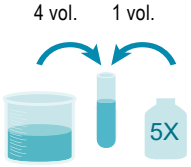
REAGENT PREPARATION

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.

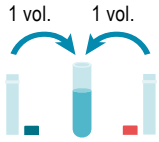
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE DILUENT, STANDARD & ANTIBODY STOCK SOLUTIONS:

1 X 96 TESTS	
IFNγ Eu Cryptate antibody	
Thaw the IFNγ Eu Cryptate antibody. Centrifuge. This stock solution can be frozen and stored at ≤-60°C.	
IFNγ XL antibody	
Thaw the IFNγ XL antibody. Centrifuge. This stock solution can be frozen and stored at ≤-60°C.	
IFNγ Standard	
Reconstitute the IFNγ standard with distilled water. Volume of reconstitution is indicated on the vial label. Due to the stability of the IFNγ, it is mandatory to prepare the standard curve just before the assay. Any remaining reconstituted analyte should be discarded.	
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.	

TO PREPARE WORKING ANTIBODY SOLUTIONS:

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

1 X 96 TESTS	
IFNγ Eu Cryptate antibody	
Dilute the stock solution (thawed reagent) of IFNγ Eu Cryptate-antibody with detection buffer #3: Add 200µL of detection buffer directly in the thawed Eu Cryptate-antibody stock solution.	
IFNγ-XL antibody	
Dilute the stock solution (thawed reagent) of IFNγ XL antibody with detection buffer #3: Add 200µL of detection buffer directly in thawed XL antibody stock solution.	
Antibody mix	
Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents: e.g. 210 µL of XL antibody + 210 µL of Eu Cryptate antibody.	

TO PREPARE WORKING STANDARD SOLUTIONS:

- Each well requires 16 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 IFNγ, it is mandatory to prepare the standard curve just before the assay. Any remaining reconstituted analyte should be discarded.
- 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:

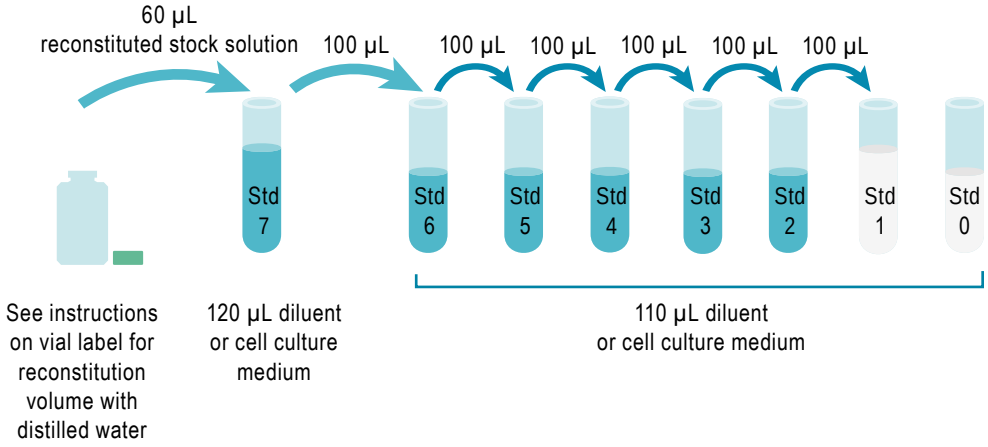
1. Reconstitute the standard vial with the volume indicated on the vial label using distilled water.

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: 4000 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 110 µL of diluent or cell culture medium into each vial from Std 6 to Std 0
- Add 100 µL of standard to 110 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	4000 pg/mL
Standard 6	100 µL Standard 7 + 110 µL diluent	1904.8 pg/mL
Standard 5	100 µL Standard 6 + 110 µL diluent	907 pg/mL
Standard 4	100 µL Standard 5 + 110 µL diluent	431.9 pg/mL
Standard 3	100 µL Standard 4 + 110 µL diluent	205.7 pg/mL
Standard 2	100 µL Standard 3 + 110 µL diluent	97.9 pg/mL
Standard 1	100 µL Standard 2 + 110 µL diluent	46.6 pg/mL
Standard 0	110 µL diluent	0

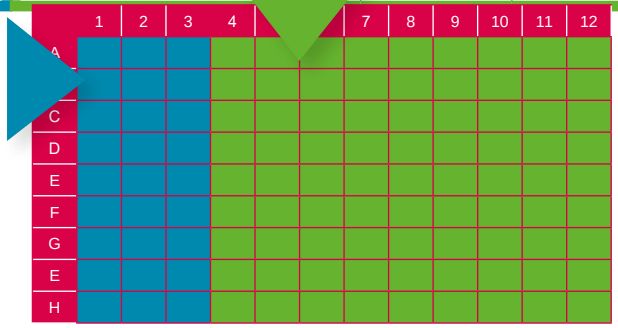
TO PREPARE SAMPLES:

- Each well requires 16 µL of sample.
- Just after their collection, put 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 16 µL of each IFNγ standard (Std 0 - Std 7) into each standard well.	Dispense 16 µL of each sample into each sample well.
Step 2	Dispense 4 µL of pre-mixed IFNγ antibodies working solution into all wells.	
Step 3	Seal the plate and incubate at room temperature.	
Step 4	Remove the plate sealer and read on an HTRF® compatible reader.	

	1	2	3	4	5	6
A	16 µL Std 0 4 µL pre-mixed IFNγ antibodies	Repeat Well A1	Repeat Well A1	16 µL Sample 1 4 µL pre-mixed IFNγ antibodies	Repeat Well A4	Repeat Well A4
B	16 µL Std 1 4 µL pre-mixed IFNγ antibodies	Repeat Well B1	Repeat Well B1	16 µL Sample 2 4 µL pre-mixed IFNγ antibodies	Repeat Well B4	Repeat Well B4
C	16 µL Std 2 4 µL pre-mixed IFNγ antibodies	Repeat Well C1	Repeat Well C1	16 µL Sample 3 4 µL pre-mixed IFNγ antibodies	Repeat Well C4	Repeat Well C4
D	16 µL Std 3 4 µL pre-mixed IFNγ antibodies	Repeat Well D1	Repeat Well D1	16 µL Sample ... 4 µL pre-mixed IFNγ antibodies	Repeat Well D4	Repeat Well D4
E	16 µL Std 4 4 µL pre-mixed IFNγ antibodies	Repeat Well E1	Repeat Well E1	16 µL Sample ... 4 µL pre-mixed IFNγ antibodies	Repeat Well E4	Repeat Well E4
F	16 µL Std 5 4 µL pre-mixed IFNγ antibodies	Repeat Well F1	Repeat Well F1	16 µL Sample ... 4 µL pre-mixed IFNγ antibodies	Repeat Well F4	Repeat Well F4
G	16 µL Std 6 4 µL pre-mixed IFNγ antibodies	Repeat Well G1	Repeat Well G1	16 µL Sample ... 4 µL pre-mixed IFNγ antibodies	Repeat Well G4	Repeat Well G4
H	16 µL Std 7 4 µL pre-mixed IFNγ antibodies	Repeat Well H1	Repeat Well H1	16 µL Sample ... 4 µL pre-mixed IFNγ antibodies	Repeat Well H4	Repeat Well H4



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

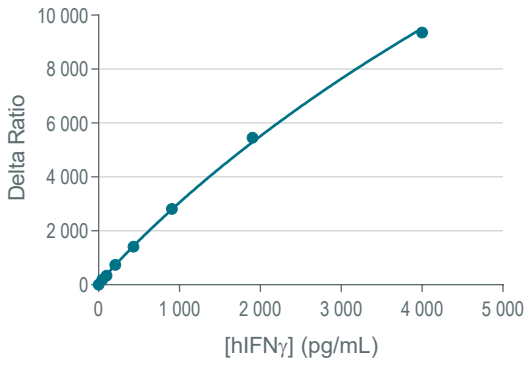
RESULTS

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 $1/y^2$)* model

* For more information about curve fitting please visit www.revvity.com

		Ratio (1)	delta R (2)	CV% (3)
Standard 0	Negative control	624	0	1%
Standard 1	46.6 pg/mL	814	190	1%
Standard 2	97.9 pg/mL	960	336	3%
Standard 3	205.7 pg/mL	1361	737	0%
Standard 4	431.9 pg/mL	2037	1413	1%
Standard 5	907 pg/mL	3432	2809	2%
Standard 6	1904.8 pg/mL	6081	5457	1%
Standard 7	4000 pg/mL	9980	9355	1%

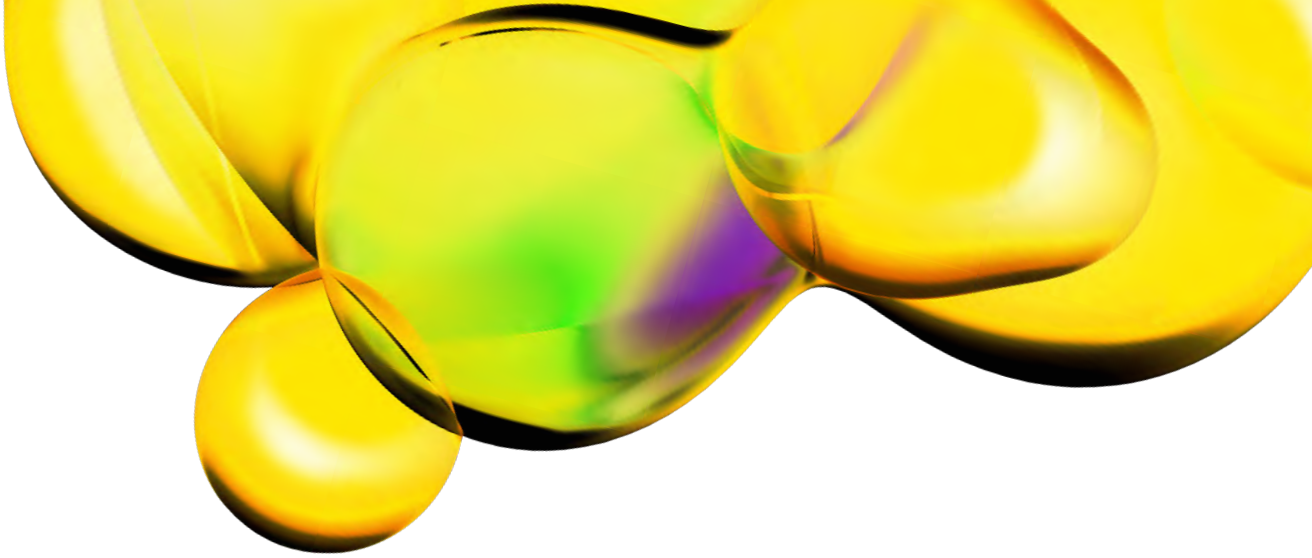


ANALYTICAL ASSAY PERFORMANCE

	Diluent	DMEM	RPMI
Assay range (pg/mL**)	21 pg/mL to 4000 pg/mL		
Limit of detection (LoD*) = Std 0 mean + 2 SD	14 pg/mL	30 pg/mL	34 pg/mL
Limit of quantification (LoQ*)	21 pg/mL		
Incubation time	at room temperature		

**NIBSC (82/587) value (IU/mL) = 0,019 x HTRF hIFNγ value (pg/mL)

*the analytical sensitivity was calculated from data obtained with an HTRF compatible reader after overnight of incubation, this may vary from one HTRF compatible reader to another.



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

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