MANUAL

HTRF Mouse IFNγ Detection Kit

Part # 62MIFNGPEG & 62MIFNGPEH

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

Revision: #08 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 mouse IFNγ assay is only intended for the quantitative measurement of IFNγ in supernatant using HTRF® technology. The assay is compatible with mouse 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 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 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).

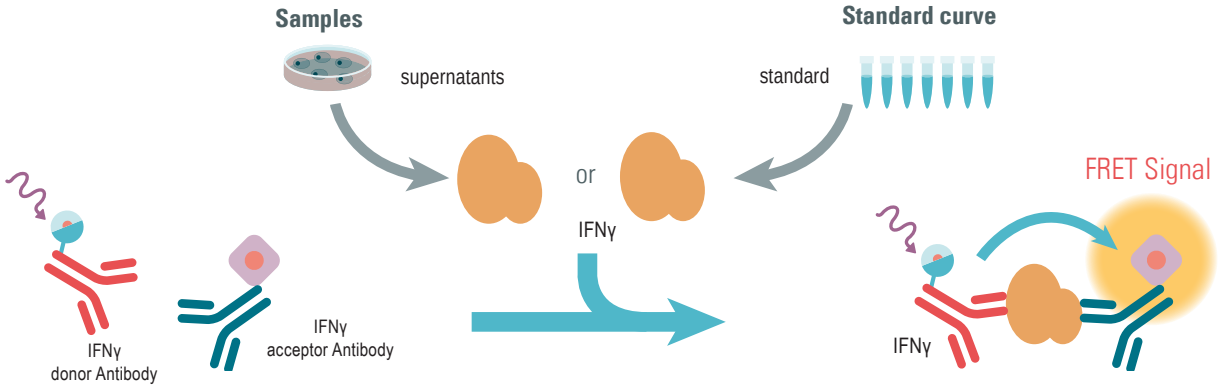
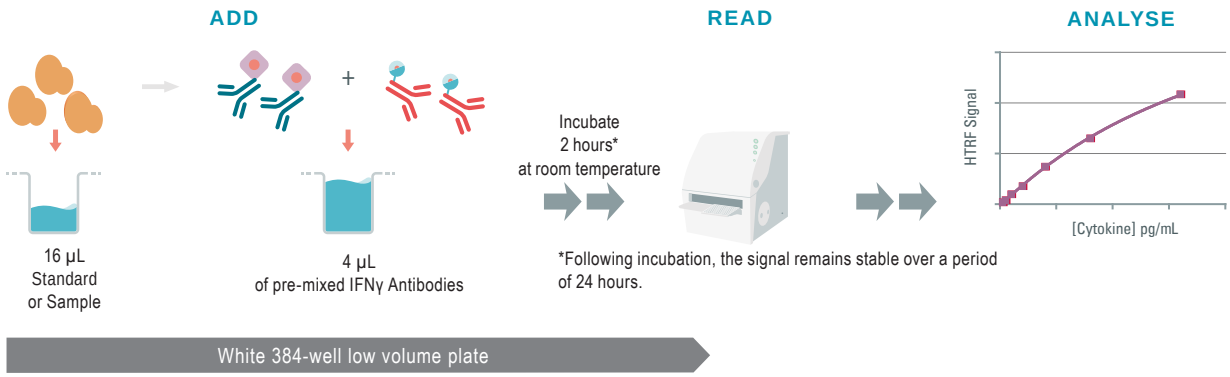


Figure 1: Principle of the HTRF IFNγ sandwich assay.

MANUAL AT A GLANCE



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 # 62MIFNGPEG	10,000 TESTS CAT # 62MIFNGPEH
IFNγ Standard Lyophilized	2 vials	2 vials
IFNγ Eu Cryptate Antibody Frozen - 20 X	1 vial - 50 µL	1 vial - 1 mL
IFNγ d2 Antibody Frozen - 20 X	1 vial - 50 µL	1 vial - 1 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

* 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.

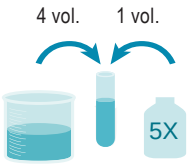
REAGENT PREPARATION

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.

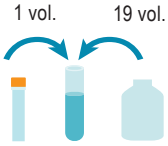
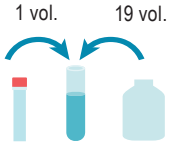
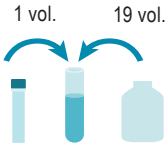
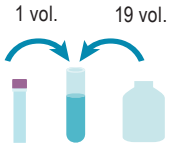
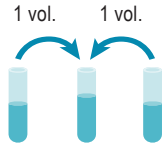
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE DILUENT, STANDARD & ANTIBODY STOCK SOLUTIONS:

500 TESTS		10,000 TESTS
IFNγ Eu Cryptate antibody		
Thaw the IFNγ Eu Cryptate antibody. Centrifuge. This 20 X stock solution can be frozen and stored at ≤-60°C.		Thaw the IFNγ Eu Cryptate antibody. Centrifuge. This 20 X stock solution can be frozen and stored at ≤-60°C.
IFNγ d2 antibody		
Thaw the IFNγ d2 antibody. Centrifuge. This 20 X stock solution can be frozen and stored at ≤-60°C.		Thaw the IFNγ d2 antibody. Centrifuge. This 20 X 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. The reconstituted standard solution can be frozen and stored at -60°C or below.		Reconstitute the IFNγ 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 IFNγ antibodies. Prepare the two antibody solutions in separate vials.

500 TESTS		10,000 TESTS	
IFNγ Eu Cryptate antibody			
Dilute 20-fold the 20 X stock solution (thawed reagent) of IFNγ 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 IFNγ Eu Cryptate antibody with detection buffer #3: e.g. 10 µL of thawed Eu Cryptate antibody stock solution + 190 µL of detection buffer).
IFNγ d2 antibody			
Dilute 20-fold the 20 X stock solution (thawed reagent) of IFNγ 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 IFNγ 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:

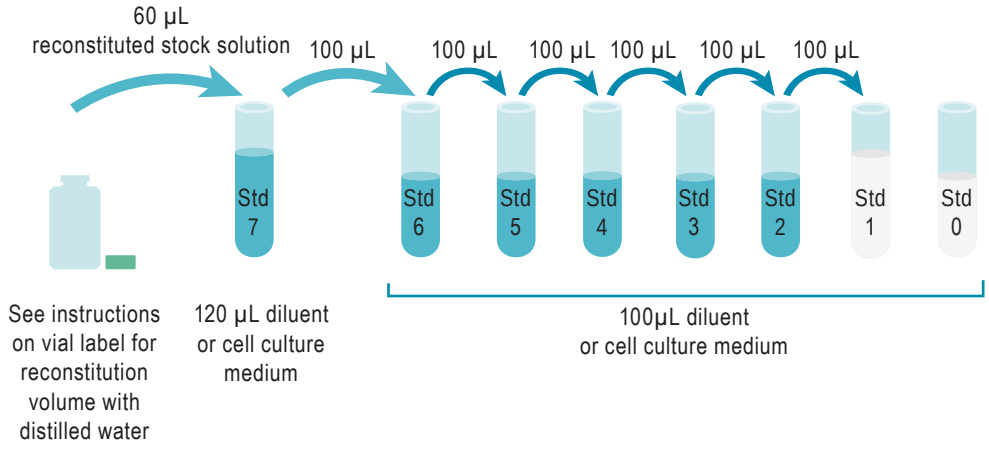
- Each well requires 16 µL of standard.
- Serially dilute the standard stock solution with diluent #5 or with the cell culture medium used to prepare your samples, supplemented with BSA or 10% FCS.
- Due to the stability of the IFNγ, it is mandatory to prepare the standard curve just before the assay.
- 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: 3000 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 100µL of diluent or cell culture medium into each vial from Std 6 to Std 0
- Add 100 µL of standard to 100µL 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	9 ng/mL
Standard 7	60 µL reconstituted standard stock solution + 120 µL diluent	3000 pg/mL
Standard 6	100 µL Standard 7 + 100µL diluent	1500 pg/mL
Standard 5	100 µL Standard 6 + 100µL diluent	750 pg/mL
Standard 4	100 µL Standard 5 + 100µL diluent	375 pg/mL
Standard 3	100 µL Standard 4 + 100µL diluent	188 pg/mL
Standard 2	100 µL Standard 3 + 100µL diluent	94 pg/mL
Standard 1	100 µL Standard 2 + 100µL diluent	47 pg/mL
Standard 0	100µL diluent	0

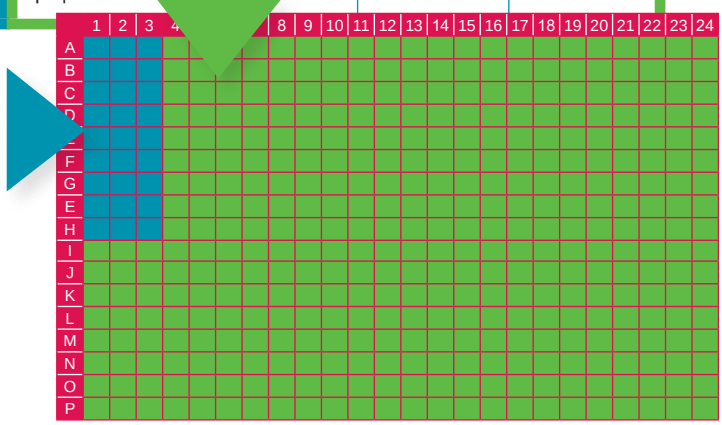
TO PREPARE SAMPLES:

- Each well requires 16 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 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 2 hours* at room temperature. *Following incubation, the signal remains stable over a period of 24 hours.	
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

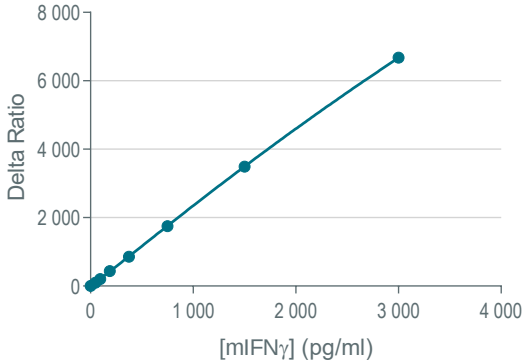
RESULTS

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	650	0	2%
Standard 1	47 pg/mL	749	99	4%
Standard 2	94 pg/mL	851	202	3%
Standard 3	188 pg/mL	1086	437	1%
Standard 4	375 pg/mL	1504	854	2%
Standard 5	750 pg/mL	2399	1750	3%
Standard 6	1500 pg/mL	4142	3492	1%
Standard 7	3000 pg/mL	7328	6679	1%



ANALYTICAL ASSAY PERFORMANCE

	Diluent	DMEM	RPMI
Assay range (pg/mL)	34 pg/mL to 3000 pg/mL		
Limit of detection (LoD*) = Std 0 mean + 2 SD	23 pg/mL	31 pg/mL	30 pg/mL
Limit of quantification (LoQ*)	34 pg/mL		
Incubation time	2 hours at room temperature		

* The analytical sensitivity was calculated from data obtained with an HTRF compatible reader after 2 hours incubation, this may vary from one HTRF compatible reader to another.

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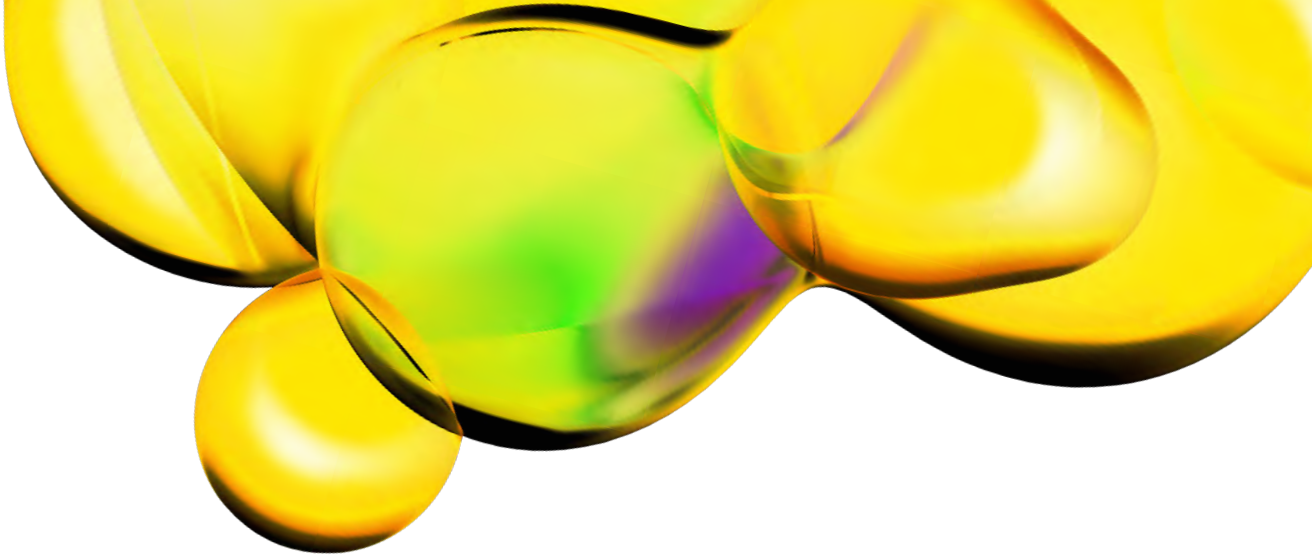
The use of the cell line will be done with appropriate safety and handling precautions to minimize health and environmental impact.



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