MANUAL

MOUSE TNFα KITS

Part # 62MTNFAPEG & 62MTNFAPEH

Test Size#: 500 tests (62MTNFAPEG), 10,000 tests (62MTNFAPEH)

Assay volume: 20 µL

Revision: #10 of April 2024 **Store at:** ≤-16°C

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

ASSAY PRINCIPLE

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

TNFα 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 TNFα present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the TNFα concentration. (Fig. 1).

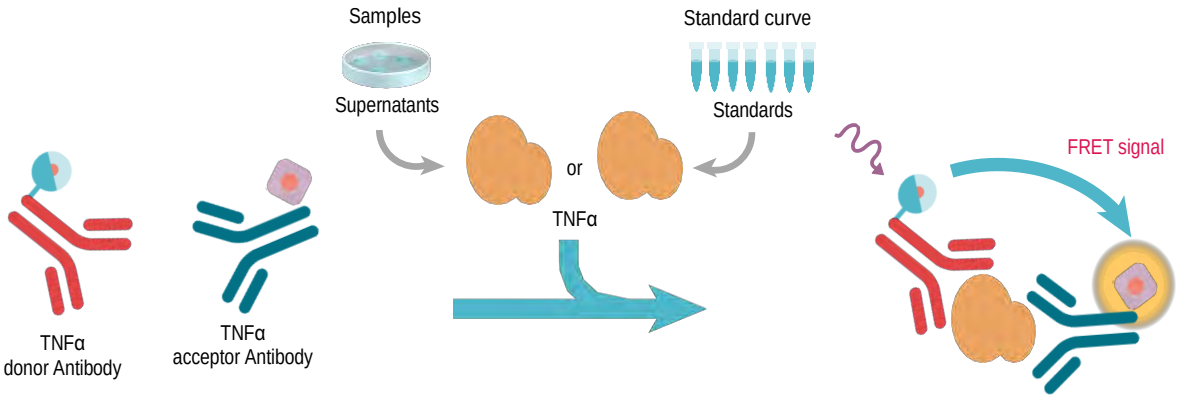
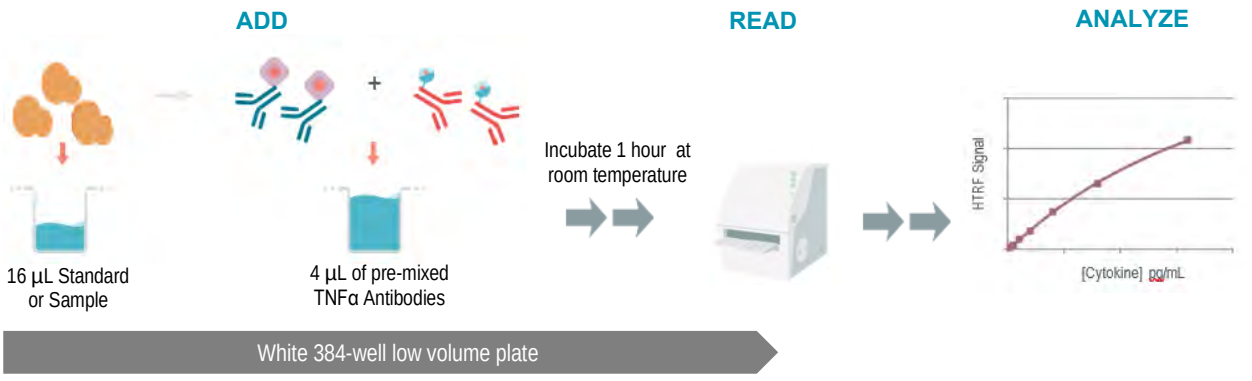


Figure 1. Principle of HTRF TNFα sandwich assay

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#62HIL12P40PEG	10,000 TESTS CAT#62HIL12P40PEH
TNFα Standard Lyophilized	2 vials	2 vials
TNFα Eu Cryptate Antobody Frozen 20X	1 vial 50 µL	1 vial 1 mL
TNFα d2 Antibody Frozen 20X	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

KIT:

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

REAGENTS:

- 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.

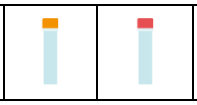
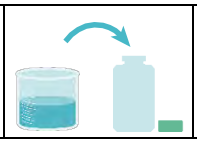
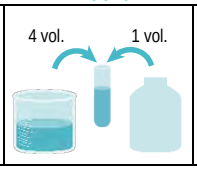
REAGENT PREPARATION

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
  - homogenise 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.

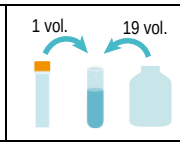
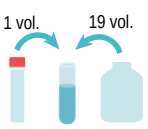
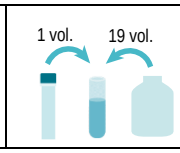
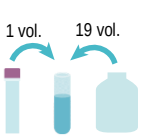
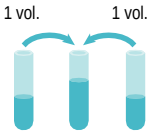
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE DILUENT, STANDARD & ANTIBODY STOCK SOLUTIONS:

500 TESTS			10,000 TESTS
TNFα Eu Cryptate antibody			
Thaw the TNFα Eu Cryptate antibody. Centrifuge. This 20X stock solution can be frozen and stored at ≤-60°C.			Thaw the TNFα Eu Cryptate antibody. Centrifuge. This 20X stock solution can be frozen and stored at ≤-60°C.
TNFα d2 antibody			
Thaw the TNFα d2 antibody. Centrifuge. This 20X stock solution can be frozen and stored at ≤-60°C.			Thaw the TNFα d2 antibody. Centrifuge. This 20X stock solution can be frozen and stored at ≤-60°C.
TNFα Standard			
Reconstitute the TNFα 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 TNFα 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 5X diluent #5 with distilled water: Homogenize the 5X 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 5X diluent #5 with distilled water: Homogenize the 5X 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 TNFα antibodies. Prepare the two antibody solutions in separate vials

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

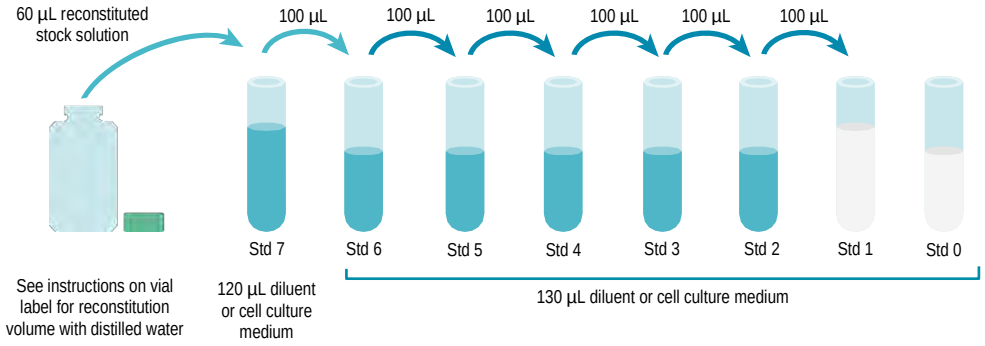
• Dilute the reconstituted standard stock solution 3-fold with diluent or with cell culture medium.

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: 6000 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 120 µL of diluent or cell culture medium into each vial from Std 6 to Std 0
- Add 100 µL of standard to 120 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	18 ng/mL
Standard 7	60 µL reconstituted standard stock solution + 120 µL diluent	6000 pg/mL
Standard 6	100 µL Standard 7 + 130 µL diluent	2608.7 pg/mL
Standard 5	100 µL Standard 6 + 130 µL diluent	1134.2 pg/mL
Standard 4	100 µL Standard 5 + 130 µL diluent	493.1 pg/mL
Standard 3	100 µL Standard 4 + 130 µL diluent	214.4 pg/mL
Standard 2	100 µL Standard 3 + 130 µL diluent	93.2 pg/mL
Standard 1	100 µL Standard 2 + 130 µL diluent	40.5 pg/mL
Standard 0	130 µL diluent	0

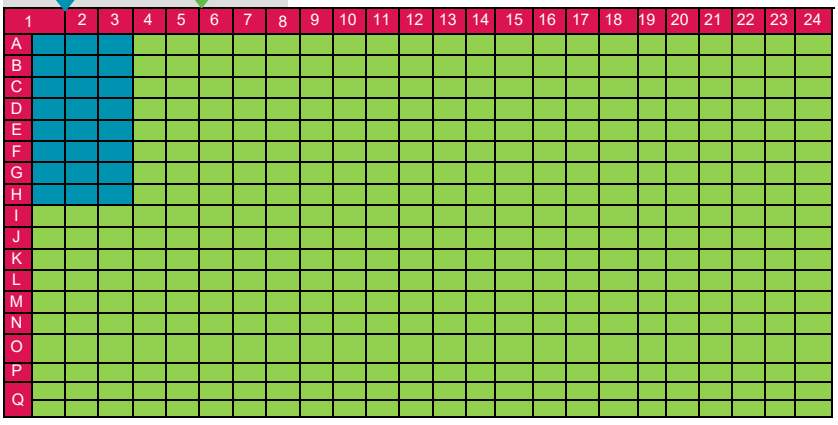
TO PREPARE SAMPLES

- 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 TNFα 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 TNFα antibodies working solution into all wells.	
Step 3	Seal the plate and incubate 1 hour 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 TNFα antibodies	Repeat Well A1	Repeat Well A1	16 µL sample 1 4 µL pre-mixed TNFα antibodies	Repeat Well A4	Repeat Well A4
B	16 µL Std 1 4 µL pre-mixed TNFα antibodies	Repeat Well B1	Repeat Well B1	16 µL sample 2 4 µL pre-mixed TNFα antibodies	Repeat Well B4	Repeat Well B4
C	16 µL Std 2 4 µL pre-mixed TNFα antibodies	Repeat Well C1	Repeat Well C1	16 µL sample 3 4 µL pre-mixed TNFα antibodies	Repeat Well C4	Repeat Well C4
D	16 µL Std 3 4 µL pre-mixed TNFα antibodies	Repeat Well D1	Repeat Well D1	16 µL sample … 4 µL pre-mixed TNFα antibodies	Repeat Well D4	Repeat Well D4
E	16 µL Std 4 4 µL pre-mixed TNFα antibodies	Repeat Well E1	Repeat Well E1	16 µL sample … 4 µL pre-mixed TNFα antibodies	Repeat Well E4	Repeat Well E4
F	16 µL Std 5 4 µL pre-mixed TNFα antibodies	Repeat Well F1	Repeat Well F1	16 µL sample … 4 µL pre-mixed TNFα antibodies	Repeat Well F4	Repeat Well F4
G	16 µL Std 6 4 µL pre-mixed TNFα antibodies	Repeat Well G1	Repeat Well G1	16 µL sample … 4 µL pre-mixed TNFα antibodies	Repeat Well G4	Repeat Well G4
H	16 µL Std 7 4 µL pre-mixed TNFα antibodies	Repeat Well H1	Repeat Well H1	16 µL sample … 4 µL pre-mixed TNFα 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

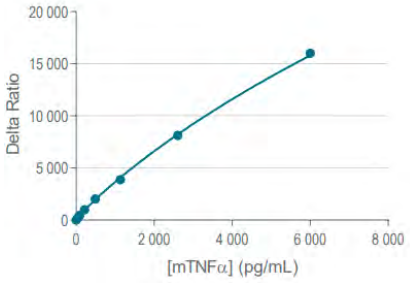
RESULTS

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/y2) model (For more information about curve fitting please visit www.revvity.com)

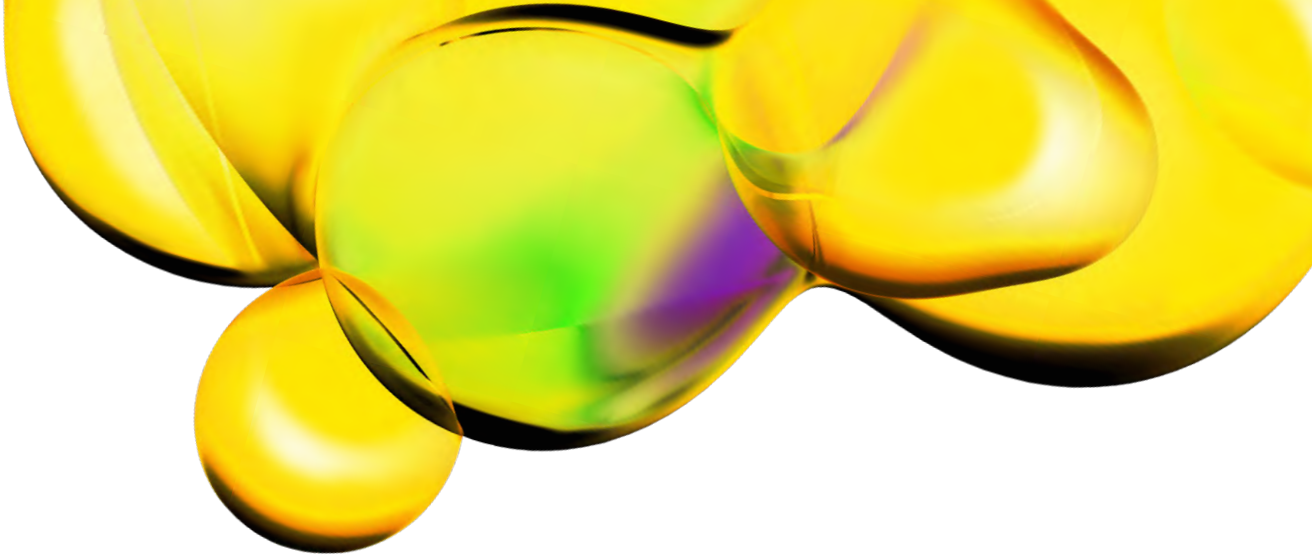
		Ratio (1)	delta R (2)	CV% (3)
Standard 0	Negative control	552	0	0%
Standard 1	40.5 pg/mL	755	203	3%
Standard 2	93.2 pg/mL	989	437	2%
Standard 3	214.4 pg/mL	1539	987	2%
Standard 4	493.1 pg/mL	2572	2020	0%
Standard 5	1134.2 pg/mL	4425	3873	4%
Standard 6	2608.7 pg/mL	8670	8118	1%
Standard 7	6000 pg/mL	16554	16002	0%



ANALYTICAL ASSAY PERFORMANCE

	Diluent	DMEM	RPMI
Assay range (pg/mL**)	20 pg/mL to 6000 pg/mL		
Limit of detection (LoD*) = Std 0 mean + 2 SD	3 pg/mL	11 pg/mL	8 pg/mL
Limit of quantification (LoQ*)	20 pg/mL		
Incubation time	1 hour at room temperature		

* The analytical sensitivity was calculated from data obtained with an HTRF compatible reader after 1 hour incubation, this may vary from one HTRF compatible reader to another.



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

www.revvity.com

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com