MANUAL

HUMAN MANF KITS

Part # 63ADK056PEG & 63ADK056PEH

Test size#: 500 tests (63ADK056PEG) and 10,000 tests (63ADK056PEH) - assay volume: 20 µL

Revision: #06 of March 2024

Store at: -16°C or below (63ADK056PEG); -16°C or below (63ADK056PEH)

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of human mesencephalic astrocyte-derived neutrophic factor (Human MANF) in cell supernatants and serum and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, Human MANF is detected in a sandwich assay by using anti Human MANF antibody labeled with Europium cryptate (donor), and anti Human MANF antibody labeled with d2 (acceptor).

When the dyes are in close proximity, 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). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the Human MANF concentration.

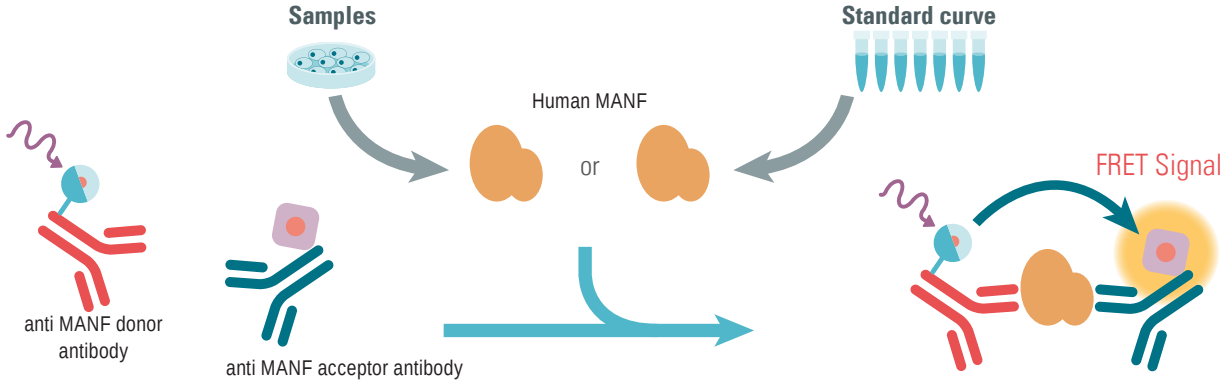
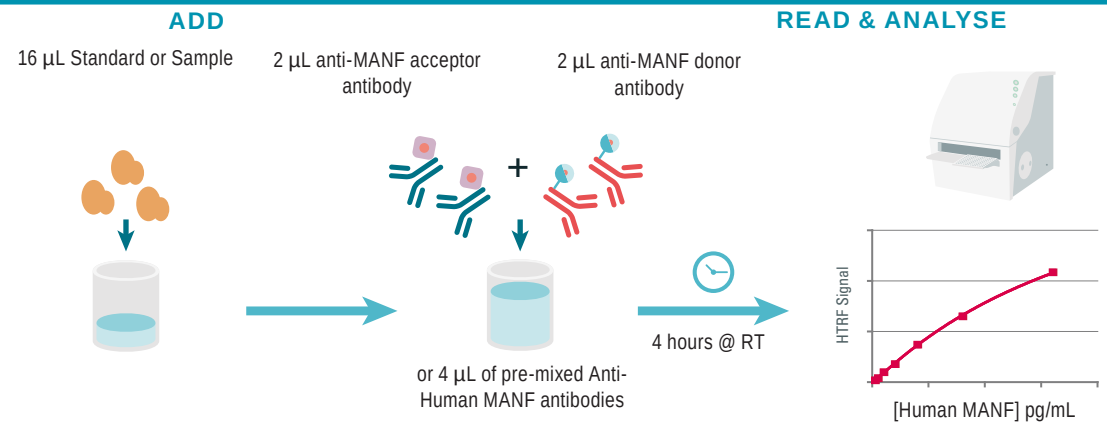


Figure 1: Principle of HTRF Human MANF sandwich assay.

MANUAL AT A GLANCE



Make sure to use the set-up for Eu Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 63ADK056PEG	10,000 TESTS * CAT # 63ADK056PEH
Human MANF Standard Frozen	1 vial - 10 µL 200 µg/mL	1 vial - 10 µL 200 µg/mL
Human MANF Eu Cryptate Antibody	1 vial - 20 µL Frozen - 50X	1 vial - 0.4 mL Frozen - 50X
Human MANF d2 Antibody	1 vial - 20 µL Frozen - 50X	1 vial - 0.4 mL Frozen - 50X
Diluent ** ready-to-use	1 vial 20 mL	1 vial 20 mL
Detection buffer *** ready-to-use	1 vial 2 mL Detection Buffer #3	1 vial 50 mL Detection buffer #3

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume.. Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** Medium like cell culture medium can be an alternative to the diluent.
*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. Make sure the setup for Eu Cryptate is used.

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

- Small volume (SV) detection microplates - Use white plate only.

For more information about microplate recommendations, please visit our website at: www.revvity.com

STORAGE AND STABILITY

Store the kit at -16°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Detection buffer is shipped frozen, but can be stored at 2-8°C in your premises.

If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below .

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, allow them to warm up to room temperature for at least 30 mins before use
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Human MANF standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

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

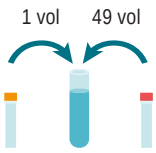
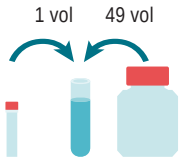
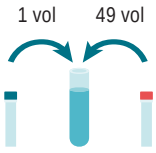
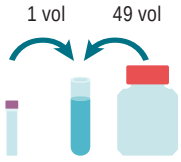
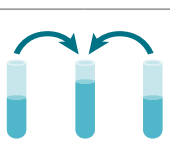
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 63ADK056PEG			10,000 TESTS KIT - 63ADK056PEH
Anti-Human MANF Eu Cryptate antibody			
Thaw the Human MANF Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the Human MANF Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
Anti-Human MANF d2 antibody			
Thaw the Human MANF d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the Human MANF d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
Human MANF Standard			
Thaw the Human MANF standard solution in order to obtain a 200 µg/mL stock solution. Mix gently.			Thaw the Human MANF standard solution in order to obtain a 200 µg/mL stock solution. Mix gently.
Diluent			
The diluent is ready-to-use.			The diluent is ready-to-use.
Detection buffer			
The Detection buffer is ready-to-use.			The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 2 µL of Human MANF-Eu Cryptate Antibody and 2 µL of Human MANF-d2 Antibody.

Prepare the two antibody solutions in separate vials.

500 TESTS KIT - 63ADK056PEG			10,000 TESTS KIT - 63ADK056PEH
Human MANF Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of human MANF Eu Cryptate antibody with the Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 20 µL of Eu Cryptate antibody stock solution + 980 µL of Detection Buffer #3).			Dilute 50-fold the 50X stock solution (thawed reagent) of human human MANF Eu Cryptate-antibody with the Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 0.4 mL of Eu Cryptate antibody stock solution + 19.6 mL of Detection Buffer #3).
Human MANF d2 antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of human MANF d2 antibody with the Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 20 µL of d -antibody stock solution + 980µl of Detection Buffer #3).			Dilute 50-fold the 50X stock solution (thawed reagent) of human MANF d2 antibody with the Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 0.4 mL of d2 antibody stock solution + 19.6 mL of Detection Buffer #3).
Antibody mix			
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).			It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 16 µL of standard.
- Dilute the standard stock solution serially with diluent or in the medium used for the preparation of the samples.
- 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:

Dilute the standard stock solution 100-fold with diluent; this yields the Intermediate Standard solution #A (2, 000, 000 pg/mL). e.g: take 5 µL of standard stock solution and add it to 495 µL of diluent. Mix gently.

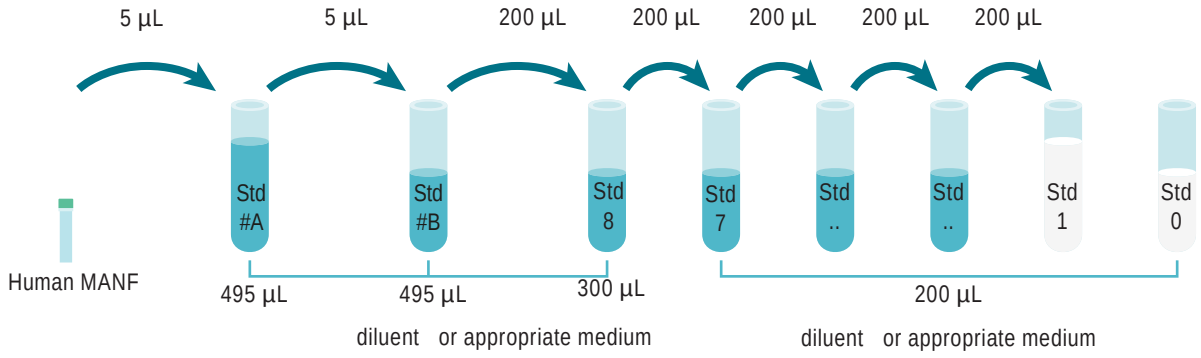
Dilute this Intermediate Standard solution #A 100-fold with diluent; this yields the Intermediate Standard solution #B (20, 000 pg/mL). e.g: take 5 µL of Intermediate Standard solution #A and add it to 495 µL of diluent. Mix gently.

Dilute the Intermediate Standard dilution #B 2.5-fold with diluent to prepare high standard (Std 8): e.g. take 200 µL of Intermediate Standard dilution #B and add it to 300 µL of diluent . Mix gently.

Use the high standard (Std 8) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 200 µL of diluent in each vial from Std 7 to Std 0.
- Add 200 µL of standard to 200 µL of diluent , mix gently and repeat the 1/2 serial dilution to make standard solutions: std7, std6, std5, std4, std3, std2, std1.

This will create 8 standards for the analyte. Std 0 (Negative control) is diluent or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	HUMAN MANF WORKING SOLUTIONS (pg/mL)
Standard Stock solution	Thawed stock solution	200,000,000
Intermediate standard solution #A	5 µL Standard stock solution + 495 µL Diluent	2,000,000
Intermediate standard solution #B	5µL Intermediate Standard Solution #A + 495 µL Diluent	20,000
Standard 8	200µL Intermediate Standard Solution #B + 300 µL Diluent	8,000
Standard 7	200 µL standard 8 + 200 µL Diluent	4,000
Standard 6	200 µL standard 7 + 200 µL Diluent	2,000
Standard 5	200 µL standard 6 + 200 µL Diluent	1,000
Standard 4	200 µL standard 5 + 200 µL Diluent	500
Standard 3	200 µL standard 4 + 200 µL Diluent	250
Standard 2	200 µL standard 3 + 200 µL Diluent	125
Standard 1	200 µL standard 2 + 200 µL Diluent	62.5
Standard 0	200 µL Diluent	0

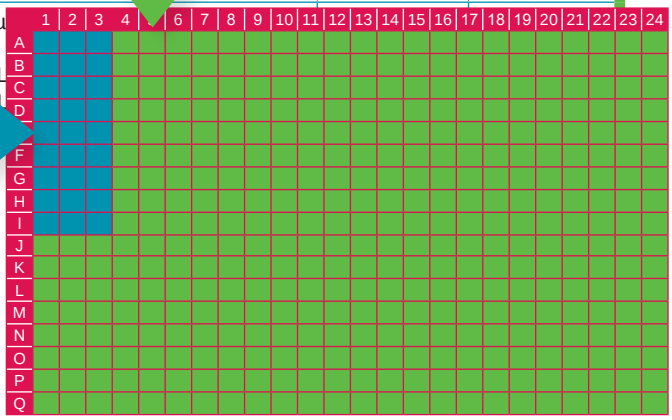
TO PREPARE SAMPLES:

- 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 or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 8) must be diluted diluent

ASSAY MANUAL

	Standard (Std 0 - Std 8)	Samples
Step 1 	Dispense 16 µL of each Human MANF standard (Std 0 - Std 8) into each standard well	Dispense 16 µL of each sample into each sample well
Step 2 	Add 2 µL of Human MANF d2 antibody working solution to all wells	
Step 3 	Add 2 µL of Human MANF Eu Cryptate antibody working solution to all wells	
Step 4 	Seal the plate and incubate 4 hours @ RT	
Step 5	Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	16 µL Std 0 (Negative control) 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well A1	Repeat Well A1	16 µL Sample 1 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well A4	Repeat Well A4
B	16 µL Std 1 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well B1	Repeat Well B1	16 µL Sample 2 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well B4	Repeat Well B4
C	16 µL Std 2 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well C1	Repeat Well C1	16 µL Sample 3 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	16 µL Std ... 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well D1	Repeat Well D1	16 µL Sample ... 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	16 µLStd ... 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well E1	Repeat Well E1	16 µL Sample ... 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	16 µL Std ... 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well F1	Repeat Well F1	16 µL Sample ... 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	16 µL Std ... 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well G1	Repeat Well G1	16 µL Sample ... 2 µL Human MA 2 µL Human MAN yptate	Repeat Well G4	Repeat Well G4
H	16 µL Std ... 2 µL Human MANF-d2 2 µL Human MANF-Eu Cryptate	Repeat Well H1	Repeat Well H1	16 µ 2 µL		



DATA REDUCTION

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

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

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control (Standard 0) plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample - Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

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

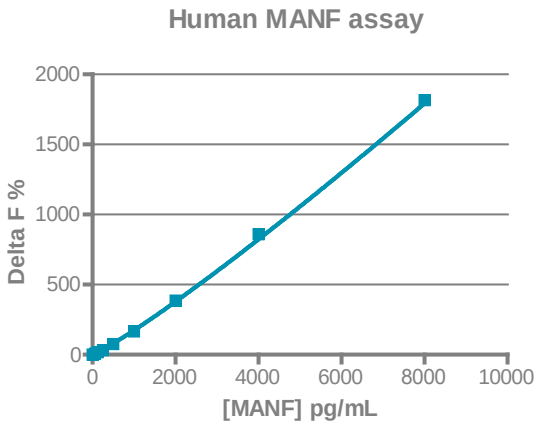
RESULTS

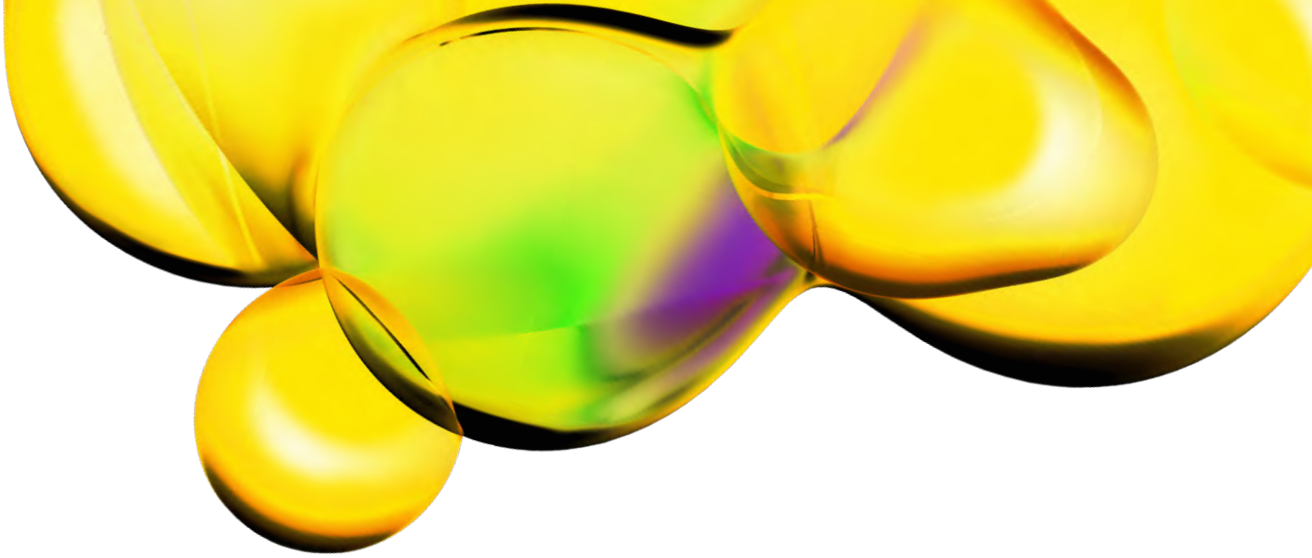
This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

The assay standard curve is created by plotting delta F% versus the analyte concentration:

	Ratio (1)	CV (2)	Delta F% (3)
Standard 0 - Negative control	393	4.0%	0%
Standard 1 - 62.5 pg/mL	425	0.2%	8%
Standard 2 - 125 pg/mL	464	3.5%	18%
Standard 3 - 250 pg/mL	521	0.8%	33%
Standard 4 - 500 pg/mL	688	0.7%	75%
Standard 5 - 1,000 pg/mL	1,044	5.4%	165%
Standard 6 - 2,000pg/mL	1,911	0.7%	386%
Standard 7 - 4,000 pg/mL	3,774	2.5%	859%
Standard 8 - 8,000 pg/mL	7,534	0.5%	1,815%





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