MANUAL

Technology: HTRF® Pathway Readout

HTRF Tau aggregation Detection Kit

Part number	6FTAUPEG	6FTAUPEH
Test size	500 tests	10,000 tests

Storage: ≤-60°C

Version: 05 **Date:** March 2024

ASSAY PRINCIPLE

This assay is intended for detection of human TAU protein aggregation using the HTRF® technology.

As shown in the diagram to the right, aggregated TAU protein is detected using one specific monoclonal antibody, labelled either with Tb-Cryptate (donor) or 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 (665nm).

The antibody labelled with d2 or Tb binds to TAU protein, when TAU protein aggregates the antibody labelled with d2 or Tb come then to a close proximity generating FRET. Signal intensity is proportional to the number of aggregates formed.

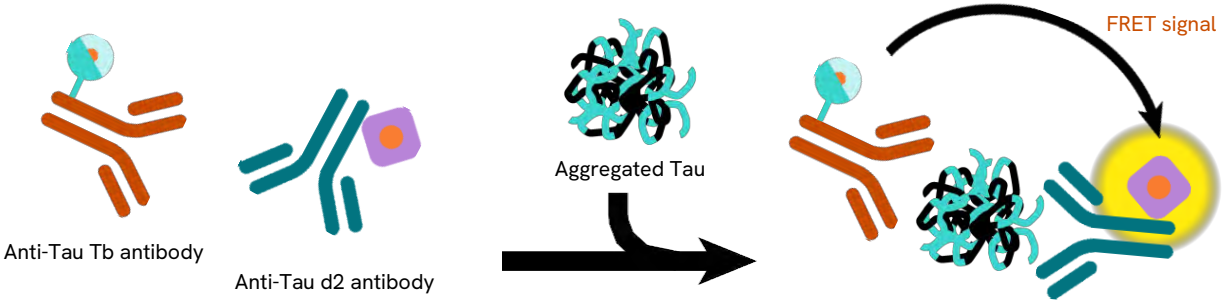
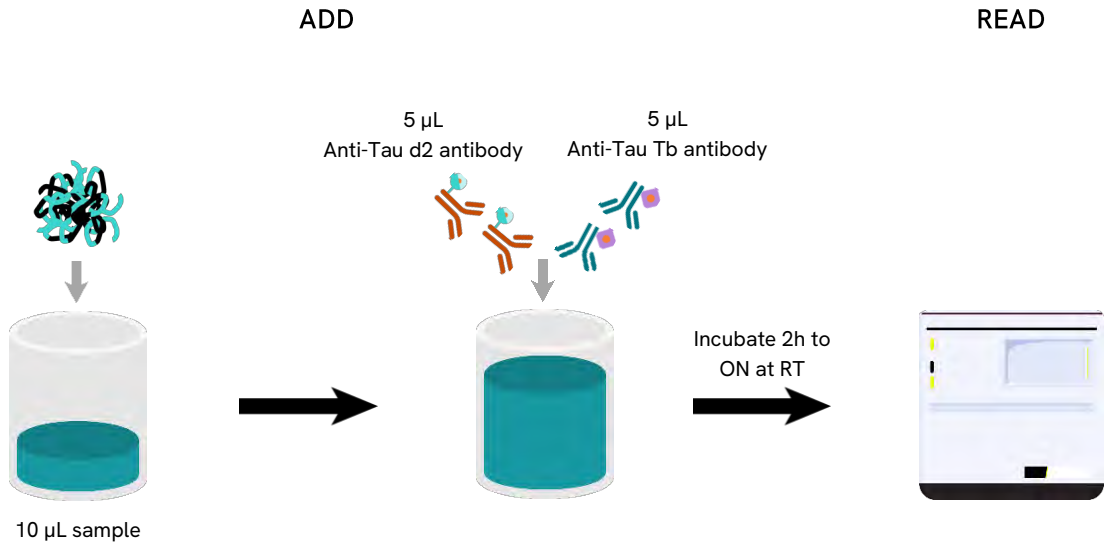


Figure 1: Principle of HTRF sandwich assay.

PROTOCOL AT A GLANCE



For HTRF certified reader

For more information about HTRF® compatible readers and for set-up recommendations, please visit our website.

MATERIAL PROVIDED

KIT COMPONENTS	STORAGE	500 TESTS			10,000 TESTS		
Positive Control	≤-60°C		green cap	50 µL/vial		green cap	1 x 50 µL/vial
Anti –human TAU Ab-d2 conjugate	≤-20°C		blue cap	50 µL/vial		purple cap	1000 µL/vial
Anti-human TAU Ab-Tb^{3+}- Cryptate-conjugate	≤-20°C		orange cap	50 µL/vial		red cap	1000 µL/vial
Diluent	+4°C to -20°C*		white cap	20mL/vial		white cap	5 X 20mL/vial
Blocking reagent Stock solution 100X	+4°C to -20°C*		purple cap	0.3 mL/vial		purple cap	3 x 2 mL/vial
Lysis buffer Stock solution 4X	+4°C to -20°C*		transparent cap	4 x 2mL/vial		white cap	130 mL/vial

* Detection buffer is shipped frozen, but can be stored at 2-8°C in your premises.

Purchase separately

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

REAGENT PREPARATION

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Tb-Cryptate antibodies will impair the assay quality.

For an accurate quantitative determination of sample, dilution must be carried out with the medium used for preparing the samples.

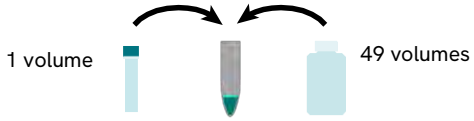
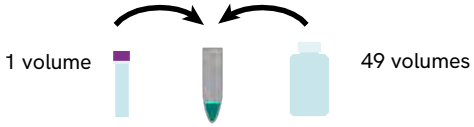
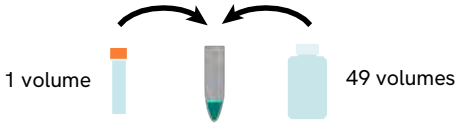
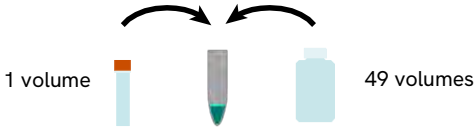
Standard and conjugates may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended to dispense remaining stock solutions of standard and conjugates into disposable plastic vials for storage at –20°C or below. Be careful, working solution preparation may differ between the 500 and the 10,000 data point kits.

- Thaw all reagents at room temperature, allow them to warm up (caution: take thawing time for buffers into account).
- Prepare the working solutions from stock solutions by following the instructions below.

Preparation of conjugate working solutions

Determine the amount of conjugate needed for the experiment. Each well requires 5µL of conjugate.

Be careful, the dilution of Anti-human TAU-d2 conjugate was changed!!!

500 TESTS KIT	10,000 TESTS KIT
Anti-human TAU-d2 conjugate	
	
Prepare a 50X diluted solution using the diluent: e.g. take 50 µL of conjugate stock solution and add it to 2450 µL of diluent.	Prepare a 50X diluted solution using the diluent: e.g. take 1000 µL of conjugate stock solution and add it to 49 mL of diluent.
Anti-human TAU-Tb^{3+}-Cryptate-conjugate	
	
Prepare a 50X diluted solution using the diluent: e.g. take 50 µL of conjugate stock solution and add it to 2450 µL of diluent.	Prepare a 50X diluted solution using the diluent: e.g. take 1000 µL of conjugate stock solution and add it to 49 mL of diluent.

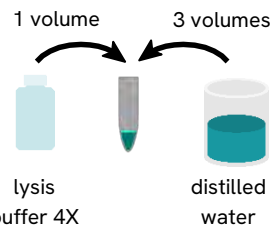
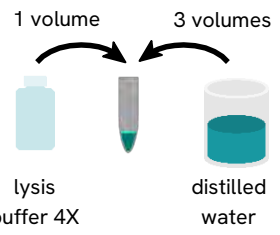
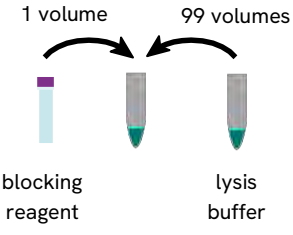
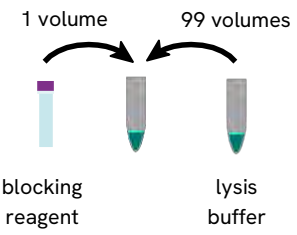
To prepare supplemented lysis buffer

Prepare the required amount of supplemented lysis buffer before running the assay, working solutions are stable for 2 days at 2-8°C.

Supplemented Lysis buffer 1X for two-plate assay manual on adherent cells

Determine the amount of supplemented lysis buffer needed for the experiment. Each well requires generally 50 µL of supplemented lysis buffer.

Prepare a lysis buffer solution 1X and then dilute the blocking reagent stock solution 100-fold with this lysis buffer 1X.

TWO-PLATE PROTOCOL ON SUSPENSION CELL & ONE-PLATE ASSAY PROTOCOL			
500 TESTS KIT & 10,000 TESTS KIT			
Preparation of lysis buffer 1X		Preparation of supplemented lysis buffer 1X	
500 tests	10,000 tests	500 tests	10, 000 tests
			
Dilute the “lysis buffer 4X” 4-fold with distilled water to prepare lysis buffer 1X. e.g. take 1.25 mL of lysis buffer 4X and add it to 3.75 mL of distilled water. Mix gently.		Dilute the “blocking reagent” 100-fold with “Lysis buffer 1X”. e.g. take 0.05 mL of “Blocking reagent stock solution” and add it to 4.95 mL of lysis buffer 1X. Mix gently.	

Preparation of sample tips

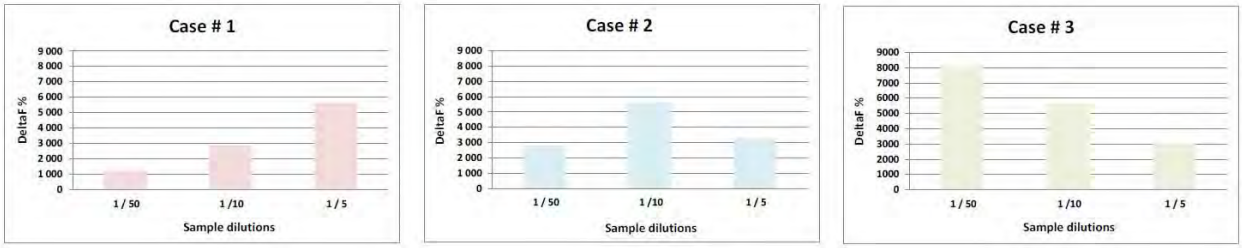
Determine how many samples and replicates to be tested.
Each well requires 10 µL of sample.
We recommend to test samples straight and a minimum of two dilutions.

	DILUTION	PREPARATION
Samples stock solution	Straight	/
Dilution 1	1/5	20 µL stock solution + 80 µL diluent
Dilution 2	1/50	10 µL dilution 1 + 90 µL diluent

Tech tips for dilution samples preparation

Case study

Depending on the sample concentration, an optimal dilution needs to be done to make sure to be in the linear part of the curve and avoid the hook effect (too high aggregate concentration capture all antibody conjugates leading to a plateau and a decrease of signal).
For example, dilution from 1/5, 1/10, 1/50 have been done on transgenic mouse brain extract (with mutated TAU).



Case #1

The signal increases: no need to make further dilutions

Case #2

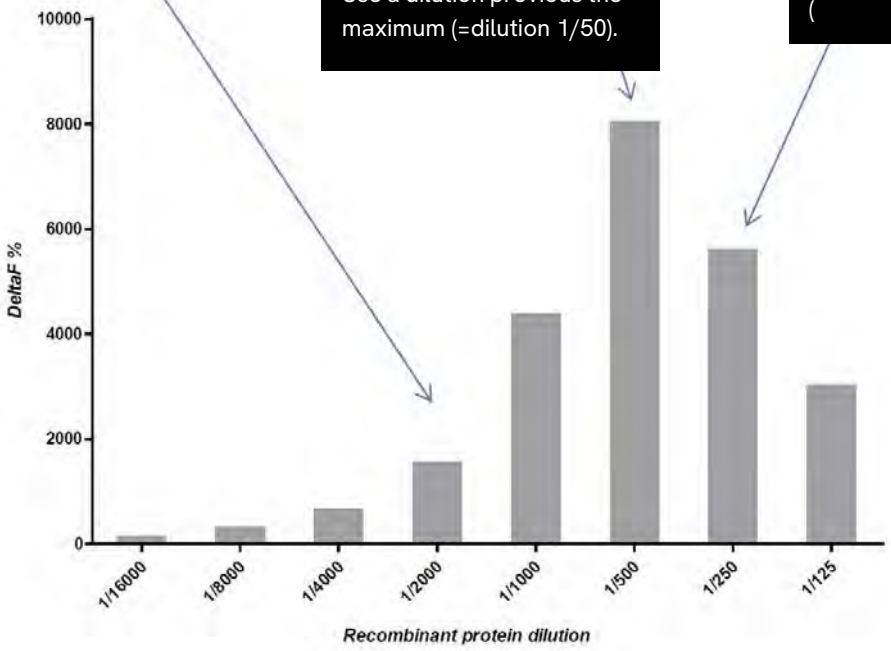
The signal increases until a maximum and decreases.

Use a dilution previous the maximum (=dilution 1/50).

Case #3

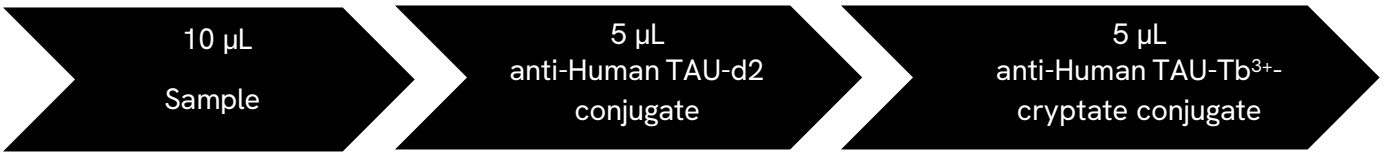
The signal decreases

=> Hook effect need to make further dilutions
(f /)



ASSAY MANUAL

Dispense the reagents in the following order:



Please Note: It is possible to pre-mix the two conjugates just before dispensing and add 10µl of this mix.

→ Cover the plate with a plate sealer.
→ Incubate 2h (if the detection is not sensitive enough, it is better to incubate 20h).
→ Remove the plate sealer and,
→ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on an HTRF® compatible reader.

For more information about HTRF® compatible readers, please visit our website.

	Assay controls			
	Negative control	Cryptate control	Diluent control	Sample / Standard
	Used to calculate the delta F%	Used to check the Cryptate signal at 620 nm	Used to check background fluorescence	
Sample / Standard	-	-	-	10 µL
Diluent	10 µL	15 µL	20 µL	-
Anti-Human TAU-d2 conjugate	5 µL	-	-	5 µL
Anti-Human TAU-Tb^{3+}-cryptate conjugate	5 µL	5 µL	-	5 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 % 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 our website.

RESULTS

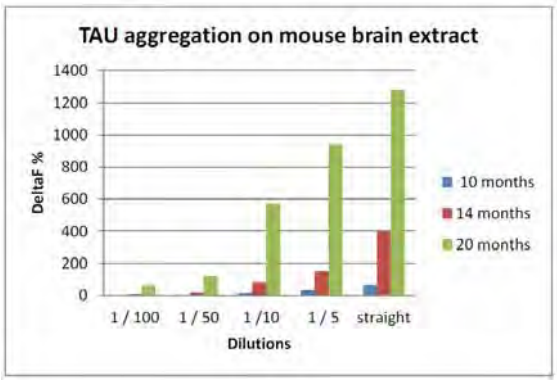
These data should be considered only as an example. Results may vary from one HTRF® compatible reader to another.

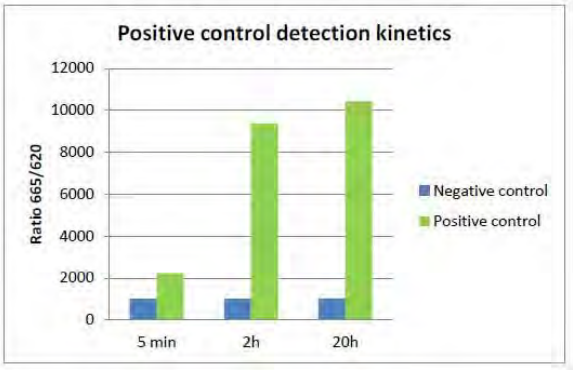
Example of TAU aggregation on triple transgenic mouse brain extract (TauPS2APP*)
(*Fiona Grueninger et al.Neurobiol. Dis. 37, 294-306)

Mouse 10 months	Ratio (1)	CV% (2)	Delta F% (3)
Negative control	1 013	1.1	/
Dilution 1/100	1 049	0.8	4
Dilution 1/50	1 069	0.3	6
Dilution 1/10	1 200	0.6	18
Dilution 1/5	1 349	6.7	33
Straight	1 671	0.6	65

Mouse 14 months	Ratio (1)	CV% (2)	Delta F% (3)
Negative control	1 013	1.1	/
Dilution 1/100	1 117	1.4	10
Dilution 1/50	1 212	0.0	20
Dilution 1/10	1 843	1.5	82
Dilution 1/5	2 540	3.4	151
Straight	5 029	0.0	397

Mouse 20 months	Ratio (1)	CV% (2)	Delta F% (3)
Negative control	1 013	1.1	/
Dilution 1/100	1 631	0.6	61
Dilution 1/50	2 248	0.3	122
Dilution 1/10	6 804	0.5	572
Dilution 1/5	10 541	0.5	941
Straight	13 979	1.4	1 280





The positive control signal (lot 01A) must be >3 over the negative control. Here is an example read on a HTRF compatible reader.

REACH European regulations and compliance
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Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

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