

## Detection of human tau protein aggregation

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- Detection and quantification of tau aggregates in brain tissue
- Kinetic of tau aggregation and dissociation
- Small sample size < 10 µl

### Introduction

The tau protein stabilizes microtubule structures in the brain. These structures are supporting the nutrient transport between neurons. Abnormal tau protein leads to collapse of structure and transport – plaques will be developed. This happens in patients that undergo neurodegeneration, e.g. in Alzheimer's. The level of a patient's tau protein can therefore be an indicator of a neurodegeneration disease state. To that end Cisbio developed a tau aggregation kit that can be applied to cell cultures, brain tissue extracts, and recombinant proteins.

### Assay Principle

Tau aggregates are measured using a sandwich immunoassay, applying an anti-tau monoclonal antibody labeled either with terbium-cryptate or d2, ensuring assay quality reproducibility and signal quality. The specific HTRF signal that is generated is proportional to the amount of tau aggregates (Fig. 1).

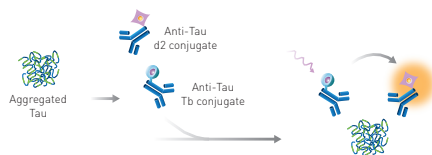


Fig. 1: HTRF® tau aggregation assay principle.

When terbium-cryptate and d2 are in close proximity, the excitation of the HTRF donor with a laser or a flash lamp will lead to an energy transfer (FRET) to the HTRF acceptor. The result is a specific FRET signal at 665 nm. At a wavelength of 620 nm the donor emission is measured.

Figure 2 shows the steps to carry out the tau aggregation assay protocol.

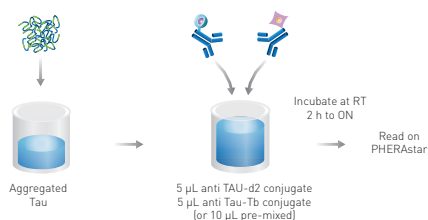


Fig. 2: HTRF® mix and measure protocol.

### Material & Methods

- TAU aggregation assay kit (#6FTAUPEG) from Cisbio including a white 384-well low volume microplate
- PHERAstar FS microplate reader from BMG LABTECH

Only 10 µl of sample is needed and given into a well of a 384-well plate. Next 5 µl anti-tau-d2 antibody and 5 µl anti-tau-tb antibody are added. After incubation of 2 hours at room temperature the HTRF signals are measured in a PHERAstar FS microplate reader.

#### Instrument settings

Detection Method:	Time-resolved fluorescence, endpoint
Optic:	Top optic
Optic Module:	HTRF 337 665 620
Integration start:	60 µs
Integration time:	400 µs
Excitation source:	Laser or flash lamp
Simultaneous dual emission:	Yes

The results of the two emission signals will be automatically converted into HTRF Ratio or DeltaF% values by the MARS Data Analysis Software.

### Results & Discussion

#### Assay specificity and linearity

Figure 3 shows that the assay can clearly distinguish between samples containing tau chemically aggregated in comparison to non aggregated tau.

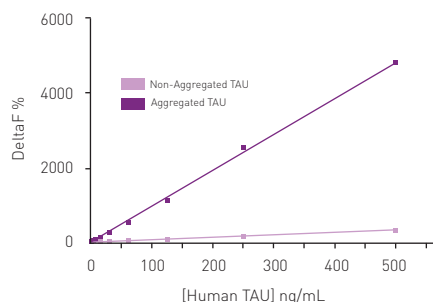


Fig. 3: DeltaF% values obtained for aggregated tau and non aggregated tau.

While non aggregated tau does not show a significant increase, HTRF values of aggregated tau samples increase with concentration. There is a linear relationship for tau aggregation.

### Kinetics of tau aggregation

Chemical aggregation was used to receive aggregates of recombinant full length human tau protein. To evaluate kinetic parameters, 5 samples were prepared in parallel and the reaction was stopped at different time points (between 1 and 24 hours) (Fig. 4).

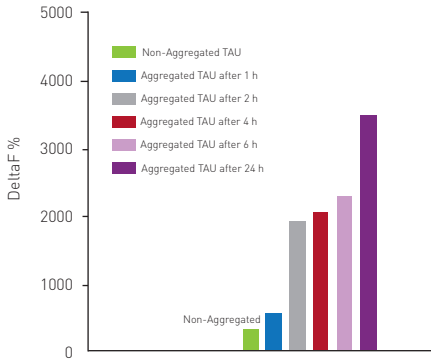


Fig. 4: Kinetics of tau aggregation.

This assay system can also be applied for monitoring a kinetic of dissociation after compound addition.

### Tau aggregation on transgenic mouse brain extracts

Tau aggregation was determined in brain extract samples from transgenic mice (Tau/PSEN2/APP). From these mice it is known that they develop neurodegeneration pathology over time (Fig. 5).

### Tau aggregation on transgenic mouse brain extracts

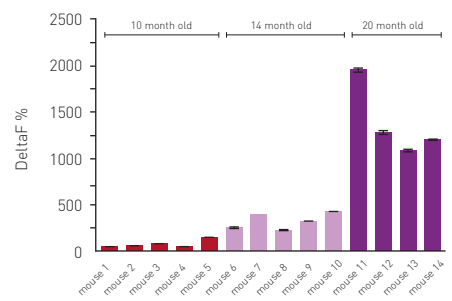


Fig. 5: Tau aggregation of transgenic mouse brain extracts.

The figure shows that not only tau aggregates in late stages can be detected with the assay (red bars). The assay is specific enough to discriminate between early stages of tau fibrillization (yellow and blue bars).

### Conclusion

Tau aggregation was successfully measured on the PHERAstar FS microplate reader. While the sample volume is very small (< 10 µl) the assay is applicable for detection of early stage fibrillization. Using the HTRF technology offers the possibility to measure a sample several times without the effect of bleaching. This property of the HTRF chemicals allow for kinetic measurements to monitor tau aggregation in real time.



PHERAstar® FS