Detection and Quantification of Tau Aggregates in Brain Tissue

Published on October 27, 2015 at 11:10 AM

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Introduction

Human brain consists of microtubule structures to support the transport of nutrients between neurons, and these structures are stabilized by the tau protein. The microtubule structures and nutrient transport will be collapsed in the presence of abnormal tau protein, leading to the development of plaques. This condition can be observed in patients suffering from neurodegenerative diseases such as Alzheimer´s.

Therefore, it is possible to know about the neurodegenerative disease state of patients from their tau protein level. In this regard, a tau aggregation kit has been developed by Cisbio for applications in recombinant proteins, brain tissue extracts, and cell cultures.

Assay Principle

A sandwich immunoassay is used to measure tau aggregates through the application of an anti-tau monoclonal antibody that is labeled either with d2 or terbium-cryptate in order to ensure signal quality and assay quality reproducibility. The specific HTRF signal produced varies in proportion to the quantity of tau aggregates, as shown in Figure 1.

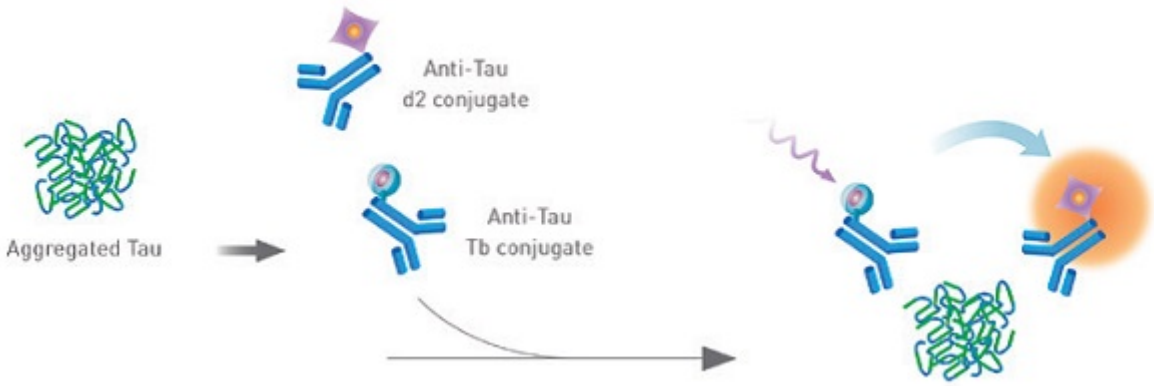

Figure 1. HTRF® tau aggregation assay principle

In the presence of closely spaced d2 and terbium-cryptate, an energy (FRET) is transferred to the HTRF acceptor when the HTRF donor is excited by a flash lamp or laser, resulting in a specific 665nm FRET signal. The measurement of the donor emission is carried out at 620nm wavelength. The steps to perform tau aggregation assay protocol are depicted in Figure 2.

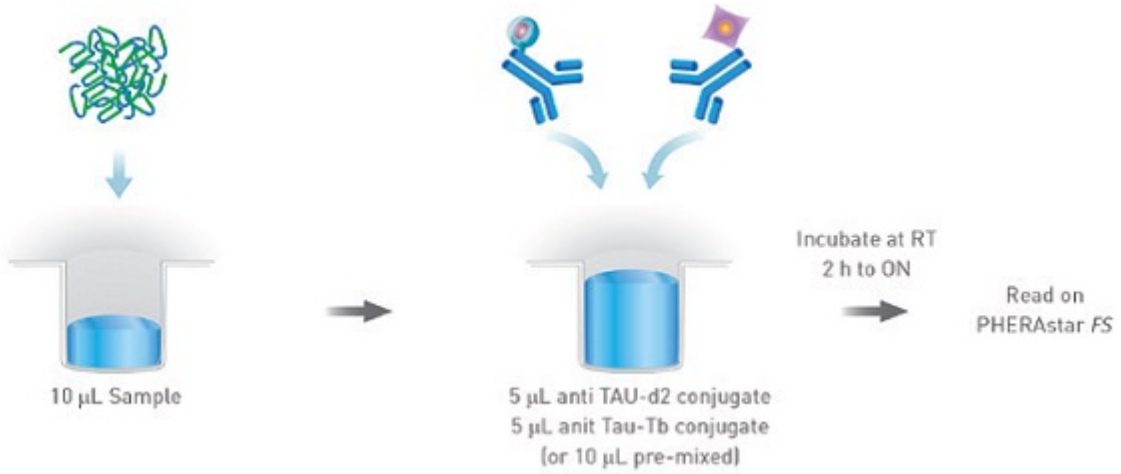

Figure 2. HTRF® mix and measure protocol

Instrumentation

Cisbio's TAU aggregation assay kit (#6FTAUPEG) equipped with a white 384-well low volume microplate and BMG LABTECH's PHERAstar FS microplate reader are used in this analysis. The instrument settings are listed in the following table:

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

Experimental Procedure

The sample volume required is 10µl, and the sample is fed into a well of a 384-well plate. This is followed by adding 5µl anti-tau-d2 antibody and 5µl anti-tau-tb antibody, and incubating the sample mixture at room temperature for 2 hours. The PHERAstar FS microplate reader is used to measure the resulting HTRF signals. The MARS Data Analysis Software automatically converts the results of the two emission signals to HTRF Ratio or DeltaF% values.

Experimental Results

Assay Specificity and Linearity

The assay is able to clearly differentiate between samples consisting of chemically aggregated tau and non-aggregated tau, as shown in Figure 3. HTRF values of aggregated tau samples significantly vary with concentration, while such a level of increase is not observed in the case of non-aggregated tau. A linear relationship is observed for tau aggregation.

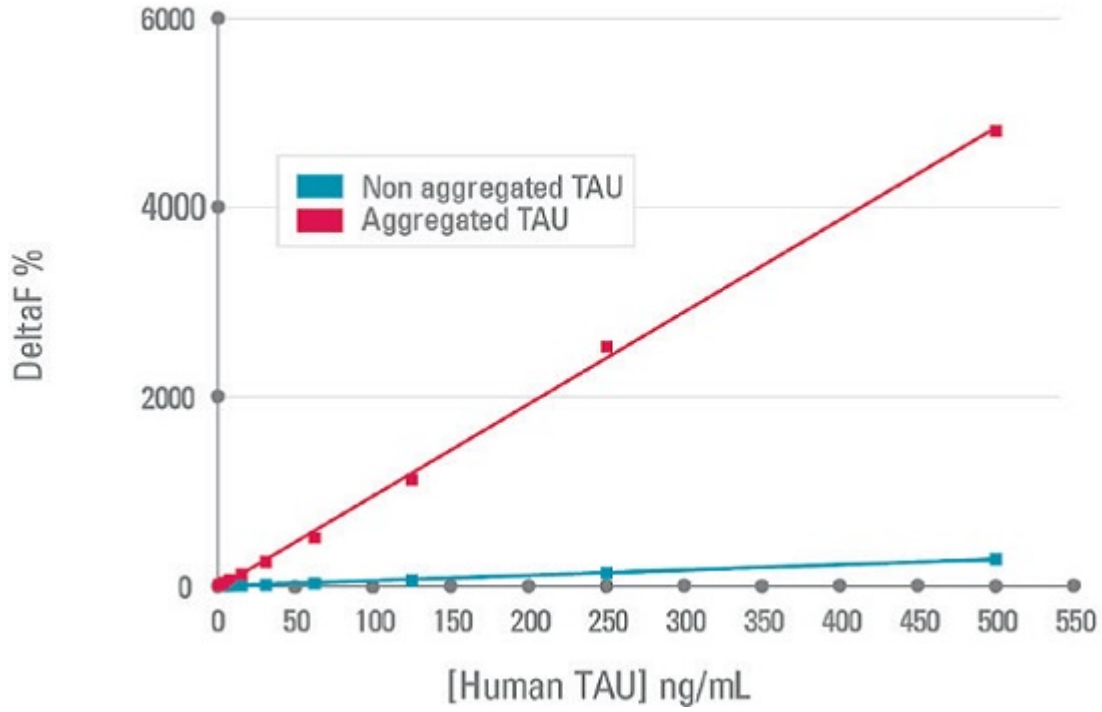

Figure 3. DeltaF% values obtained for aggregated tau and non-aggregated tau

Kinetics of Tau Aggregation

Aggregates of recombinant full-length human tau protein were obtained using chemical aggregation. Kinetic parameters were evaluated by preparing five samples in parallel and stopping the reaction at various time points, ranging from 1 to 24 hours, as shown in Figure 4.

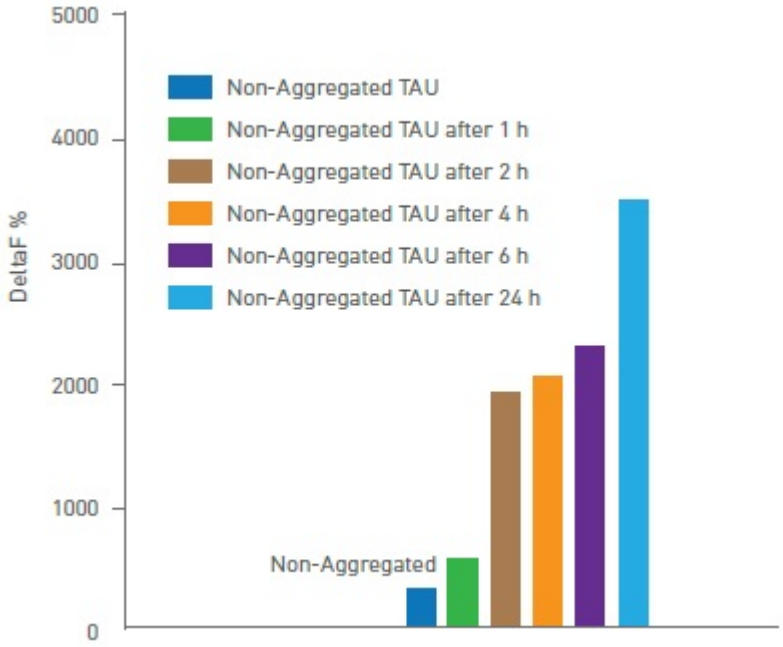

Figure 4. Kinetics of tau aggregation

A kinetic of dissociation can also be monitored following the addition of compounds, using this assay system.

Tau Aggregation on Transgenic Mouse Brain Extracts

Here, brain extract samples obtained from transgenic mice (Tau/PSEN2/APP) were analyzed for tau aggregation, showing the development of neurodegeneration pathology over time. From Figure 5, the assay not only allows detecting tau aggregates in late stages (red bars), but is also specific enough to differentiate between the early stages of tau fibrillization (yellow and blue bars).

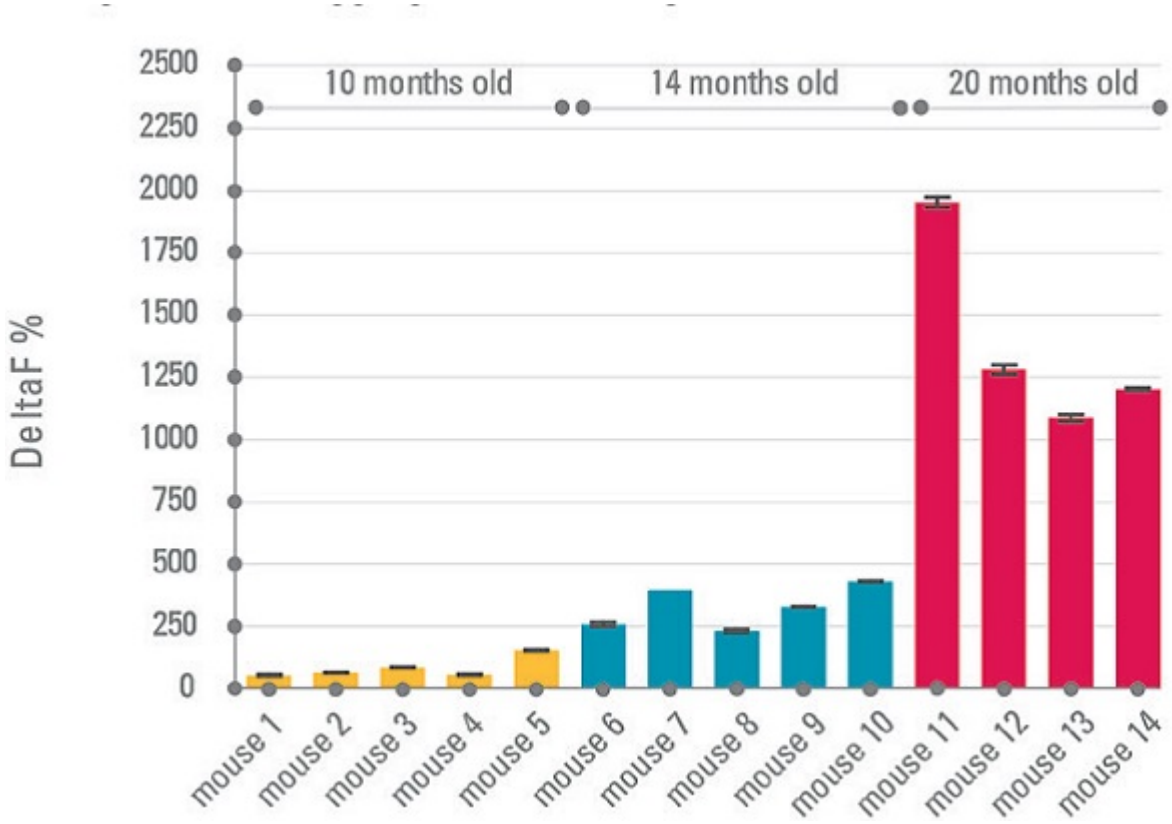

Figure 5. Tau aggregation of transgenic mouse brain extracts

Conclusion

This article has demonstrated the successful measurement of tau aggregation using the PHERAstar FS microplate reader. The assay system requires very small sample volume of less than 10µl, making it suitable to detect early stage fibrillization. A sample can be measured several times with no effect of bleaching, thanks to the HTRF technology. This capability of the HTRF chemicals enables real-time monitoring of tau aggregation by allowing kinetic measurements.

Acknowledgements

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Last updated: Oct 27, 2015 at 11:44 AM

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