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Human α-Synuclein Aggregation Kit
10.000 tests

For in vitro research use only
Reagent storage temperature: -60°C or below

www.cisbio.com

Packaging details:

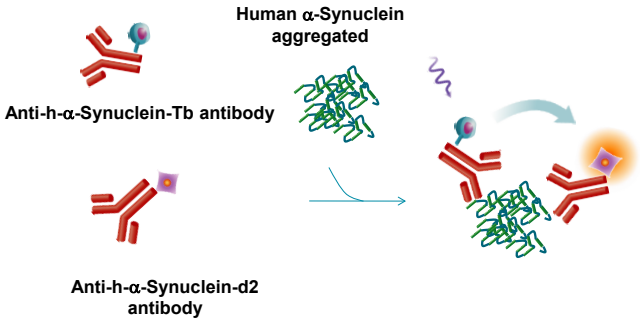
	384-well low volume plate (20 µl)
6FASYPEH	10.000 tests

Product information

Document reference: 6FASYPEH-Rev04-Sept.2019

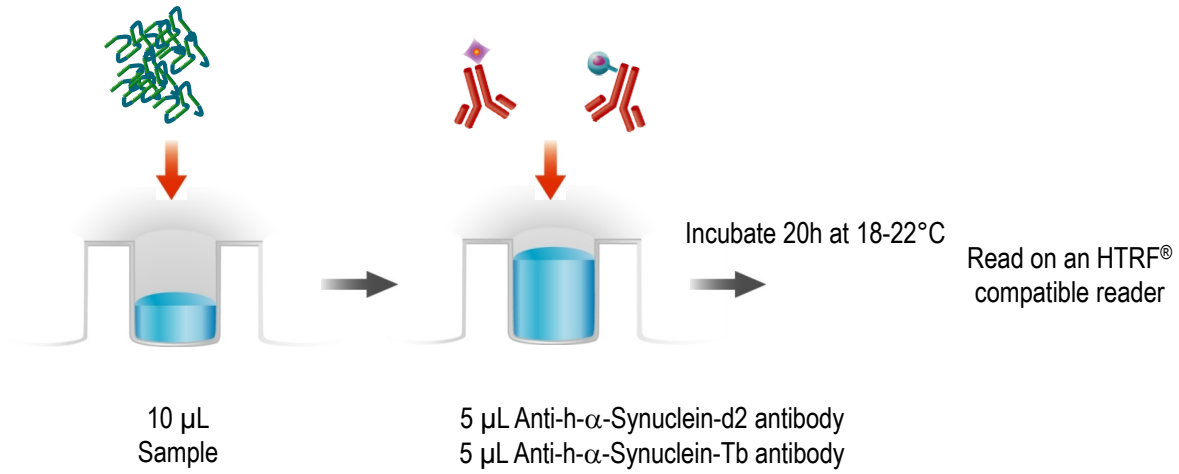
1. Assay description

This assay is intended for the detection of Human α-Synuclein aggregation using the HTRF® technology.
As shown in the diagram to the right, aggregated Human α-Synuclein 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).

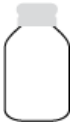


The antibody labelled with d2 or Tb binds to Human α-Synuclein. When Human α-Synuclein aggregates the antibody labelled with d2 or Tb come then into a close proximity generating FRET. Signal intensity is proportional to the number of aggregates formed.

2. Protocol at a glance



3. HTRF® reagents

	Positive Control	Anti-h-α-Synuclein-d2 antibody	Anti-h-α-Synuclein-Tb-Cryptate antibody	Lysis Buffer (stock solution 4X)	Blocking reagent (stock solution 100X)	Detection Buffer #1
						
Stock solution	3 x 50 µL/vial	1000 µL/vial	1000 µL/vial	130 mL/vial	3 x 2 mL/vial	105 mL/vial
Storage	-60°C or below	-20°C or below	-20°C or below	4°C to -20°C*	-20°C or below	4°C to -20°C*

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

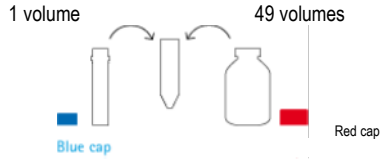
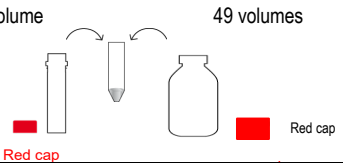
4. Reagent preparation

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 detection of sample, dilution must be carried out with the medium or lysis buffer used for preparing the samples (i.e. diluent, culture medium or any other compatible medium).
Positive control and antibodies may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended to dispense remaining stock solutions of positive control and antibodies into disposable plastic vials for storage at –60°C or below.

- 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 (§3) by following the instructions below.

4.1. Preparation of antibody working solutions

Determine the amount of antibodies needed for the experiment. Each well requires 5 µL of each antibody.

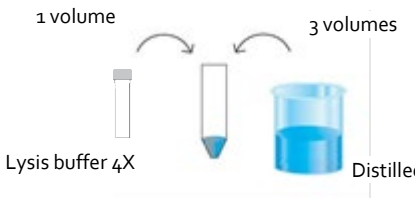
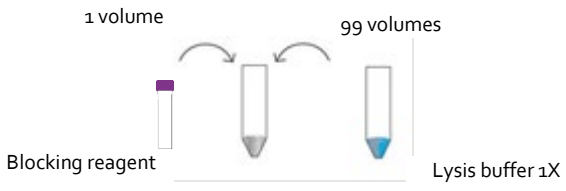
Anti-h-α-Synuclein-d2 antibody	Anti-h-α-Synuclein-Tb-Cryptate antibody
	
Prepare a 50X diluted solution using the detection buffer #1: e.g. take 1 mL of antibody stock solution and add it to 49 mL of detection buffer #1.	Prepare a 50X diluted solution using the detection buffer #1: e.g. take 1 mL of antibody stock solution and add it to 49 mL of detection buffer #1.

4.2. Preparation of sample and tips

4.2.1 Preparation of lysis buffer 1X

Prepare the required amount of lysis buffer before running the assay.
Determine the amount of lysis buffer needed for the experiment.

Prepare a lysis buffer solution. In practice:

Preparation of lysis buffer 1x	Dilution of blocking reagent
	
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.

Immediately add 50 µL of supplemented lysis buffer (1X) and incubate for at least 30 minutes to 1h at room temperature under shaking.

Use the appropriate supplemented lysis buffer and incubate at room temperature with shaking.

We recommend a time course study to determine the optimal lysis incubation time.

Lysis volume can be decreased down to 25 µL.

4.2.2 Preparation of samples

Determine how many samples and replicates to be tested.
Each well requires 10 µL of sample or positive control (ready to use).
We recommend to test a minimum of three dilutions for each sample.

	Dilution	Preparation
Dilution 1	1/4	25 µL stock solution + 75 µL Lysis buffer 1X
Dilution 2	1/40	20 µL dilution 1 + 180 µL Lysis buffer 1X
Dilution 3	1/100	40 µL dilution 2 + 60 µL Lysis buffer 1X

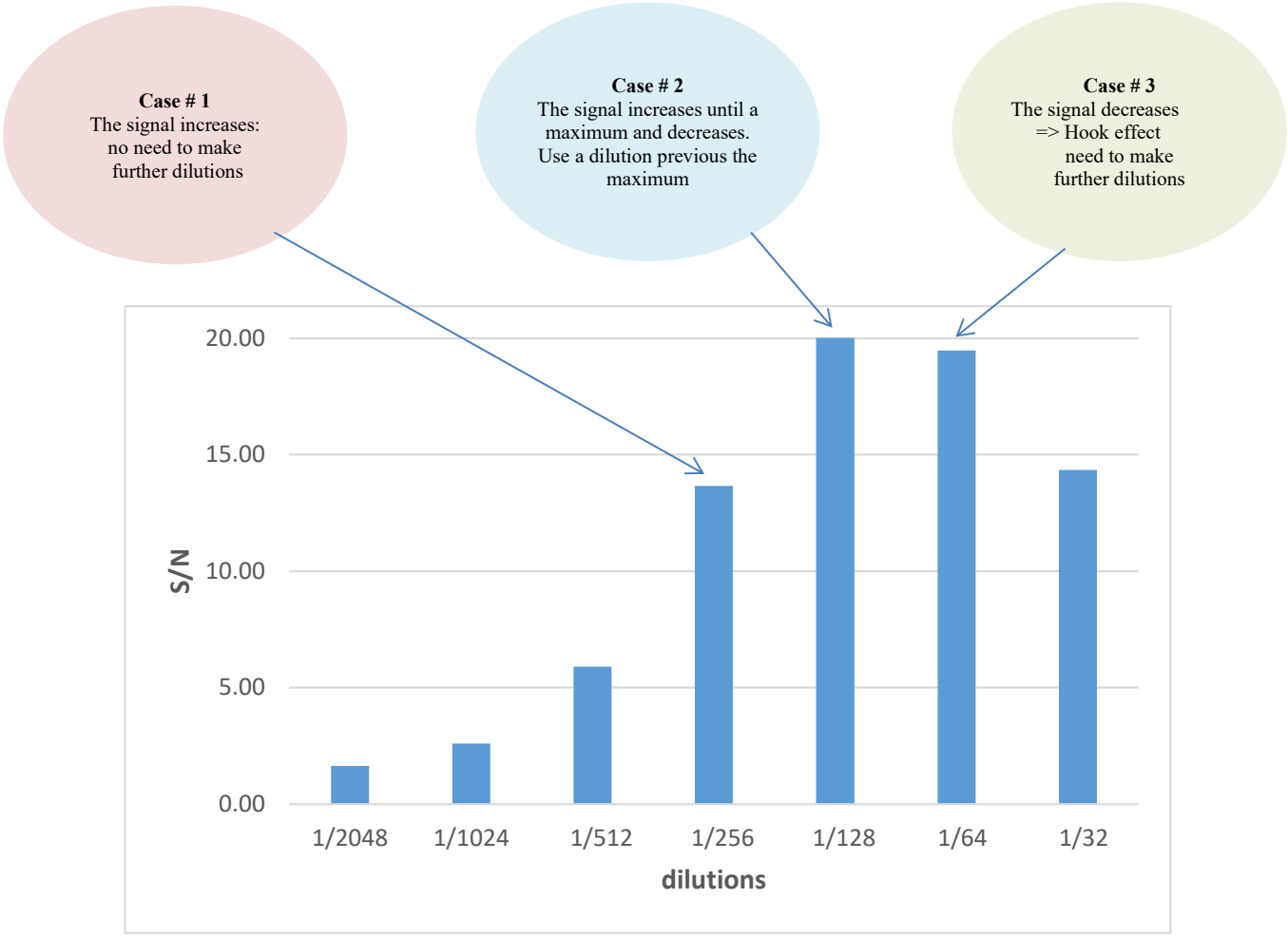
4.2.3 Tech tips for dilution samples preparation

Case study

Depending on the sample concentration, an optimal dilution needs to be done to be in the linear range of the detection and to avoid the hook effect (Too high aggregate concentrations capture all antibodies leading to a plateau and a decrease of signal).

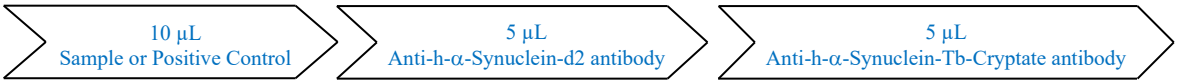
For example, several dilutions have been tested on aggregated α-Synuclein.

A signal to noise was calculated (sample ratio/negative control ratio).



5. Assay protocol

Dispense the reagents in the following order:



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

→ Cover the plate with a plate sealer.
→ Incubate 20h at 18-22°C.
→ 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 at: www.cisbio.com/compatible-readers

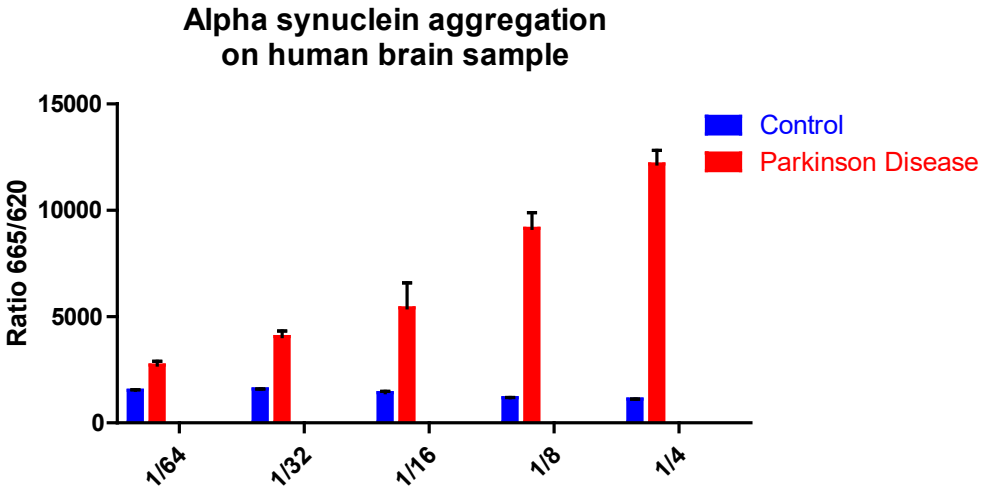
	Assay controls			
	Negative control	Cryptate control	Buffer control	Sample or Positive control
	Used to calculate the delta F %	*Used to check the Cryptate signal at 620 nm*	*Used to check background fluorescence*	
Sample or Positive control	-	-	-	10 µL
Lysis Buffer 1X	10 µL	10 µL	10 µL	-
Anti-h-α-Synuclein-d2 antibody	5 µL	-	-	5 µL
Anti-h-α-Synuclein-Tb-Cryptate antibody	5 µL	5 µL	-	5 µL
Detection Buffer #1	-	5 µL	10 µL	-

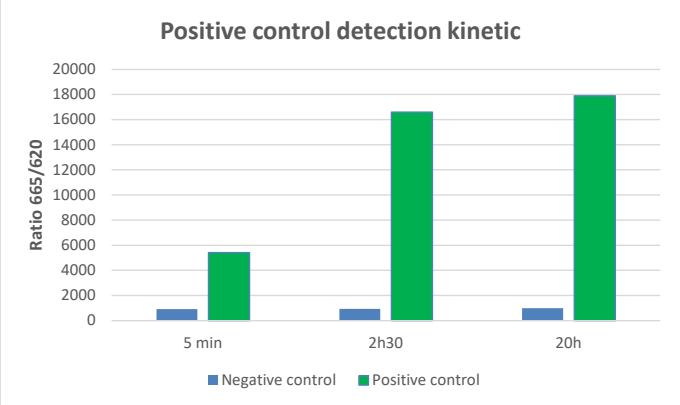
6. Data reduction

These data must not be substituted for that obtained in the laboratory and should be considered only as an example (readouts on PHERAstar^{FS}). Results may vary from one HTRF® compatible reader to another.

Example of α-Synuclein aggregation on human brain extract (from control or diagnosed with Parkinson disease)

The human brain samples were diluted in lysis buffer, and tested following the kit protocol with 20h incubation at room temperature.





The Positive control signal (lot 01A) must be > 3 times higher than the Negative control signal.

Here is an example read on PHERAstar^{FS} Laser.

Ratio	$\frac{\text{Signal}_{665nm}}{\text{Signal}_{620nm}} \times 10^4$	Ratio must be calculated for each individual well.
CV%	$\frac{\text{Standard deviation}}{\text{Mean ratio}} \times 100$	The mean and standard deviation can then be worked out from ratio replicates.
Delta F %)	$\frac{\text{Ratio}_{\text{standard or sample}} - \text{Ratio}_{\text{Negative control}}}{\text{Ratio}_{\text{Negative control}}} \times 100$	Reflects the signal to background of the assay. The negative control plays the role of an internal assay control.
For more information about data reduction, please visit our website at: www.cisbio.com/htrf-ratio-and-data-reduction		

To obtain additional information or support, please contact your technical support team (htrfservices@cisbio.com).