MANUAL

HTRF HUMAN α-SYNUCLEIN AGGREGATION Kit

Part # 6FASYPEG & 6FASYPEH

Test Size#: 500 tests (6FASYPEG) and 10,000 tests (6FASYPEH)

Assay volume: 20µL

Revision: #06 of September 2023

Store at: -60°C or below

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

ASSAY PRINCIPLE

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.

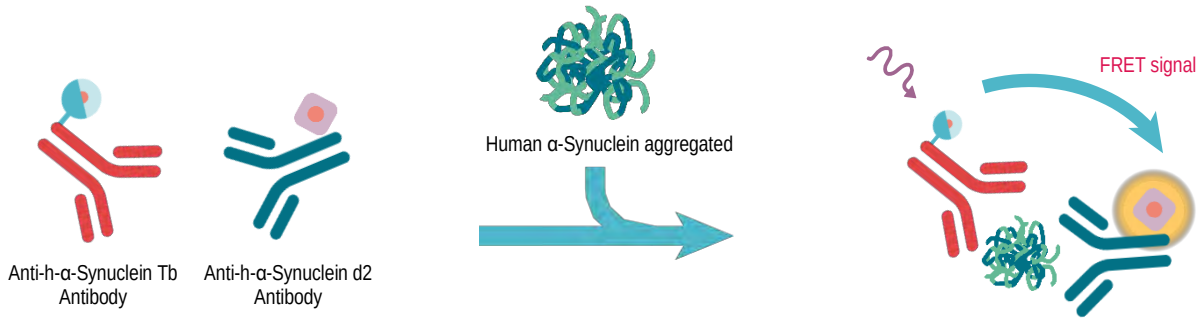
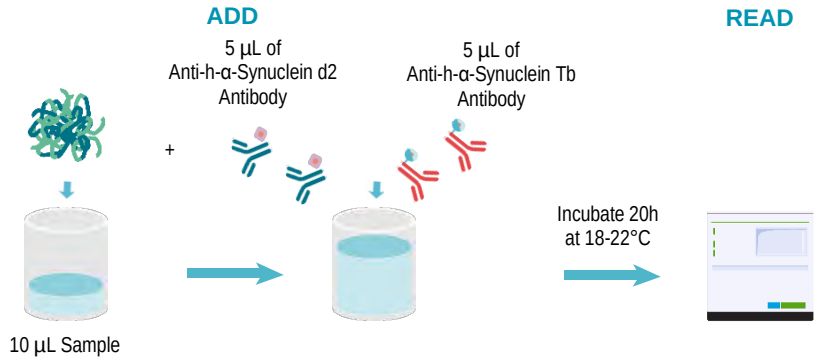


Figure 1. Principle of HTRF α-Synuclein Aggregation sandwich assay

MANUAL AT A GLANCE



Make sure to use the set-up for Eu Cryptate

MATERIALS

KIT COMPONENTS	STORAGE	500 TESTS*			10,000 TESTS*		
Positive control	≤-60°C		green cap	50 µL/vial		green cap	3 x 50 µL/vial
Anti-h-α-Synuclein-d2 antibody	≤-20°C		blue cap	50 µL/vial		blue cap	1000 µL/vial
Anti-h-α-Synuclein-Tb- Cryptate antibody	≤-20°C		orange cap	50 µL/vial		red cap	1000 µL/vial
Lysis Buffer Stock solution 4X	≤-20°C		transparent cap	4 x 2 mL/vial		white cap	130 mL/vial
Blocking reagent Stock solution 100X	≤-20°C		purple cap	300 µL/vial		purple cap	3 x 2 mL/vial
Detection Buffer #1	4°C – 20°C*		white cap	7 mL/vial		red cap	105 mL/vial

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

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

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.

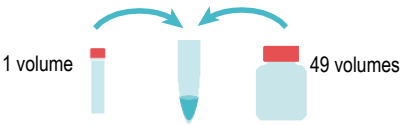
For an accurate detection of sample, dilution must be carried out with the medium or lysis buffer used for preparing the samples (i.e. diluent, culture medium or any other compatible medium).

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.

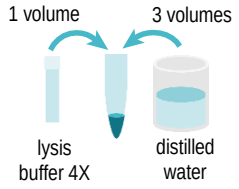
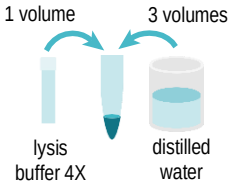
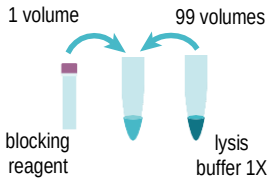
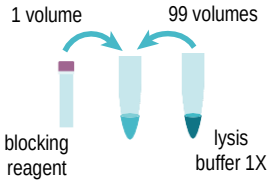
PREPARATION OF ANTIBODY WORKING SOLUTIONS

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

500 TESTS	10,000 TESTS
Anti-h-α-Synuclein-d2 antibody	
 Prepare a 50X diluted solution using the detection buffer #1: e.g. take 50 µL of antibody stock solution and add it to 2450 µL 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.
Anti-h-α-Synuclein-Tb-Cryptate antibody	
 Prepare a 50X diluted solution using the detection buffer #1: e.g. take 50 µL of antibody stock solution and add it to 2450 µL 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.

PREPARATION OF LYSIS BUFFER 1

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:

<table>
<tr><th colspan="4">500 TESTS KIT & 10,000 TESTS KIT</th></tr>
<tr><td colspan="2">Preparation of lysis buffer 1X</td><td colspan="2">Dilution of blocking reagent</td></tr>
<tr><td>500 tests</td><td>10,000 tests</td><td>500 tests</td><td>10,000 tests</td></tr>
<tr><td> 
</td><td> 
</td><td> 
</td><td> 
</td></tr>
<tr><td colspan="2">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.</td><td colspan="2">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.</td></tr>
</table>

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.

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

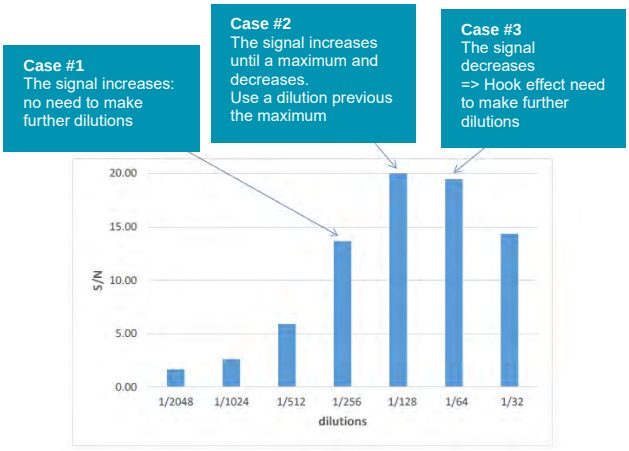
TECH TIPS FOR DILUTION SAMPLES PREPARATION

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).



ASSAY MANUAL

Dispense the reagents in the following order:

10 µL	5 µL	5 µL
Sample or positive Control	Anti-h-α-Synuclein-d2 antibody	Anti-h-α-Synuclein-Tb-Cryptate antibody

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.

	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	-

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.

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

3. Calculate the % Delta F. Reflects the signal to background of the assay. The negative control plays the role of an internal assay control.

$$\text{Delta F}(\%) = \frac{\text{Ratio}_{\text{standard or sample}} - \text{Ratio}_{\text{negative control}}}{\text{Ratio}_{\text{negative control}}} \times 100$$

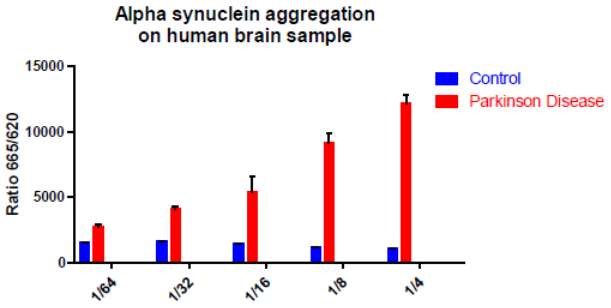
For more information about data reduction, please visit our website.

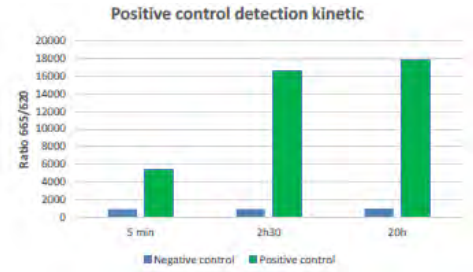
RESULTS

These data must not be substituted for that obtained in the laboratory and should be considered only as an example (readouts on PHERAstarFS). 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)

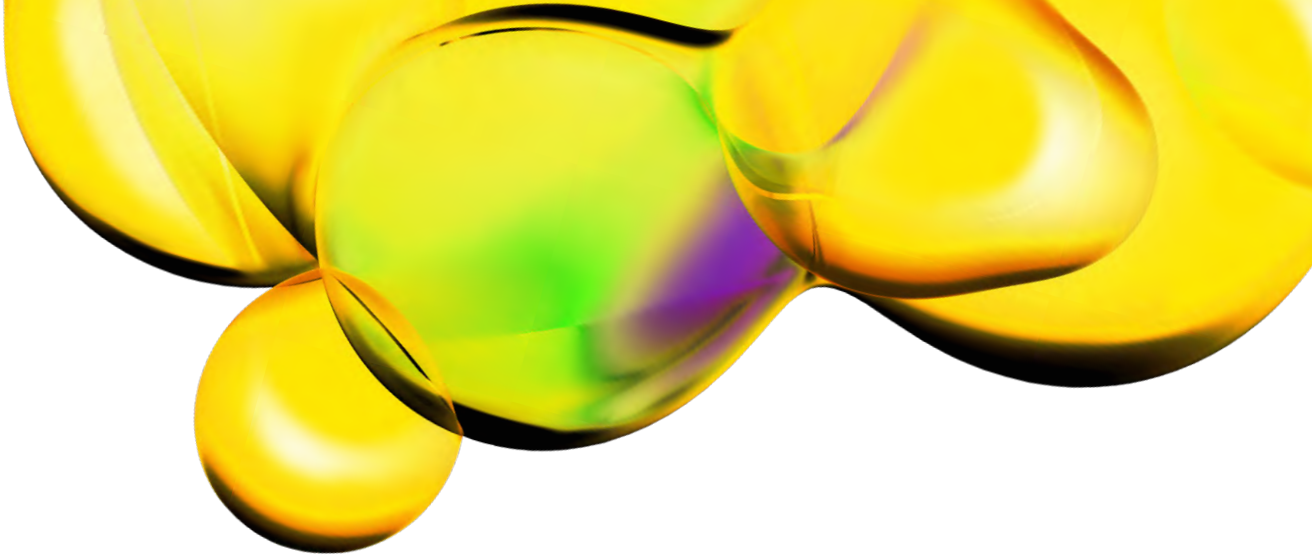
The human brain samples were diluted in lysis buffer, and tested following the kit manual with 20h incubation at room temperature.





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

Here is an example read on PHERAstarFS Laser.



Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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