Detection of phospho, total and aggregated α-Synuclein to characterize biological samples

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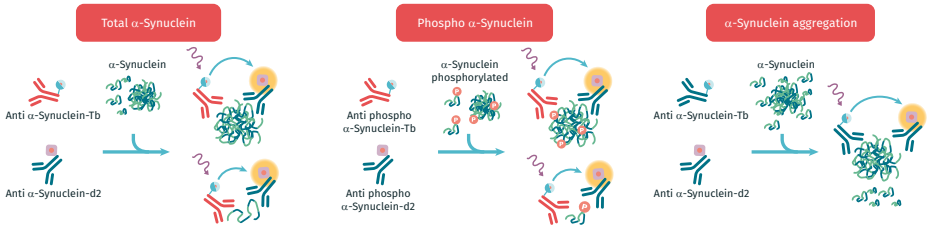
INTRODUCTION α-Synuclein is a prominent component of intracellular fibrillary aggregates in the brains of patients suffering from synucleinopathies. This protein represents a key molecular hallmark for Parkinson's disease (PD), dementia with Lewy bodies (DLB), or multiple system atrophy (MSA). The extensive phosphorylation of α-Synuclein on Ser129 is also associated with a pathological event.

Cisbio provides three kits to detect Total α-Synuclein, phosphorylated α-Synuclein (S129), or aggregated α-Synuclein on variable samples for In Vitro testing.



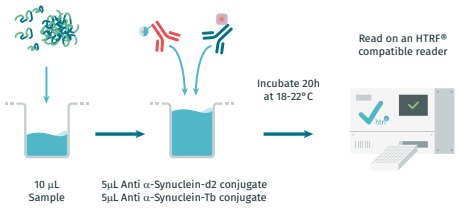
HTRF phospho, Total, and aggregation assays use 2 antibodies labeled either with donor or acceptor fluorophores.

These HTRF assays are sandwich immunoassays, which means that the intensity of the FRET signal (HTRF Ratio) is directly proportional to the concentration of the protein or aggregate in the lysates.

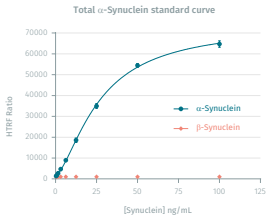
DETECTION OF TOTAL α-SYNUCLEIN ON ENDOGENOUS CELL LINES

Protocol

Endogenous cell lines were seeded at 8 million cells/well, then lysed as recommended in the kit instructions. Following a two-step protocol, Total α-Synuclein was detected by HTRF, and compared with Total protein detection using the BCA assay.

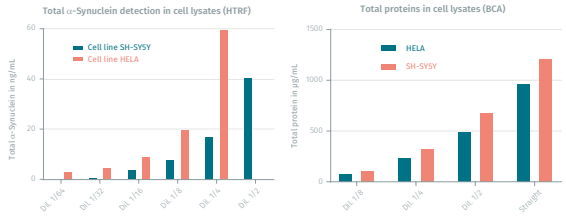


α-Synuclein standard curve



This α-Synuclein quantification assay is specific to α-Synuclein detection, and does not cross-react with β-Synuclein.

Comparison of α-Synuclein detection and Total protein detection

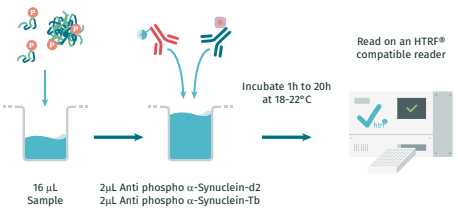


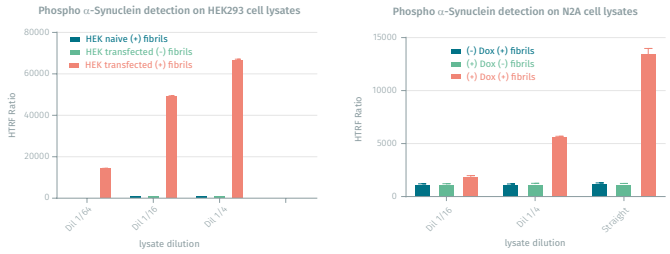
Total α-Synuclein is efficiently quantified in the different dilutions of cell lysates. The HTRF assay displays higher sensitivity and selectivity compared to the BCA detection.

DETECTION OF PHOSPHO α-SYNUCLEIN (S129) ON SEVERAL SAMPLES

Protocol

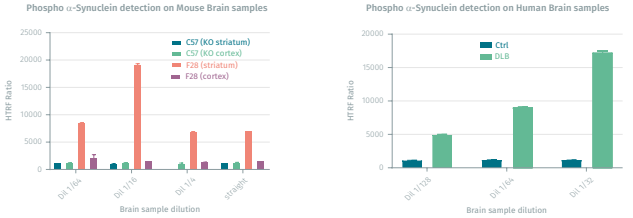
For phospho α-Synuclein (S129) detection, HEK293 over-expressing α-Synuclein and Neuroblastoma N2a cells were seeded with non phosphorylated fibrils, then lysed as recommended in the kit instructions. Following a two-step protocol, phospho α-Synuclein was detected by HTRF as described.





Only the cells over-expressing α-Synuclein (HEK293 transfected or N2A induced by doxycycline) seeded with fibrils show α-Synuclein phosphorylation.

Phospho α-Synuclein (S129) was then assessed in various mouse and human brain samples.



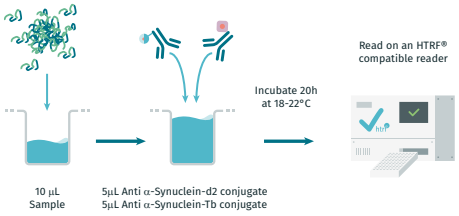
Phosphorylation (Ser 129) could be detected on transgenic mouse (F28) versus control brain samples (C57) and human DLB brain versus control brain samples.

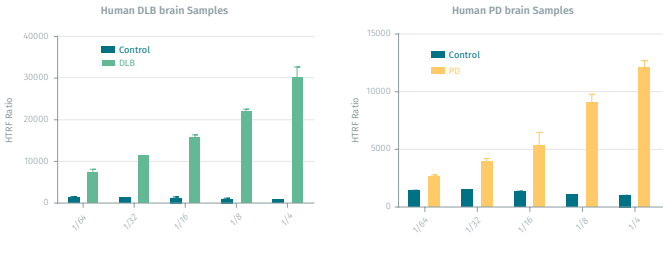
Note that for biological samples, several dilutions must be made to avoid the hook effect.

DETECTION OF AGGREGATED α-SYNUCLEIN ON HUMAN BRAIN SAMPLES

Protocol

The human brain samples were lysed as recommended in the kit instructions. Following a two-step protocol, α-Synuclein aggregates were detected by HTRF as described below.





α-Synuclein aggregation was positively detected on human PD and DLB brains versus control brain samples.

CONCLUSION Cisbio provides a versatile panel of HTRF synuclein assays enabling an in-depth investigation of various types of samples, including endogenous and over-expressing cell lines, as well as brain extracts. The lysis buffer included in these kits is used for all the assays, so it is possible to quantify the level of Total α-Synuclein, of phosphorylation, and of aggregation from the same lysate.

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