STING HTRF offer to bridge innate and adaptive immunity

cGAS-STING SIGNALING PATHWAY FROM A TO Z

By controlling the production of Interferon beta, the cGas-STING pathway acts as a bridge between innate and adaptive immunity, thereby facilitating anti-tumor immunity. Thus, the cGAS-STING pathway has emerged as a potential therapeutic target in cancer. Indeed, recent promising outcomes in eliciting anti-tumor immunity paved the way for exploring the anti-cancer potential of non-canonical cyclic di-nucleotides analogs (Ramanjulu et al, Nature 2018).

This brochure outlines the use of HTRF to investigate the cGas-Sting pathway, in the context of innate immunity, and the TCR pathway, in the context of adaptive immunity.

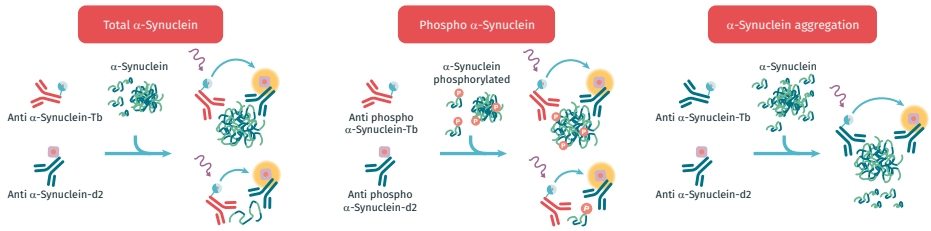
PerkinElmer® *For the Better* | cisbio

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

Karine Lafargue, Stéphane Martinez, Delphine Jaga
Cisbio Bioassays, 30200 Codolet, France

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.



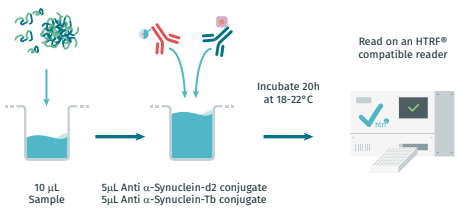
HTRF phospho, Total, and aggregation assays use 2 antibodies labeled either with donor or acceptor fluorophores.

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.

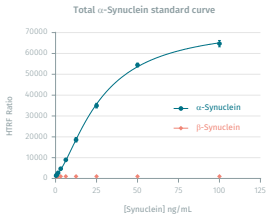
DETECTION OF TOTAL α-SYNUCLEIN ON ENDOGENOUS CELL LINES

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.

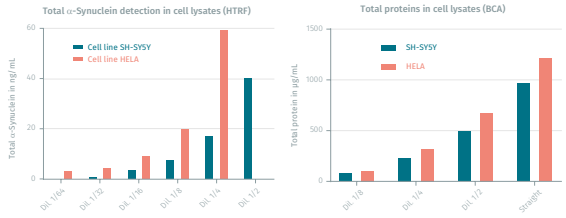


α-Synuclein standard curve



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

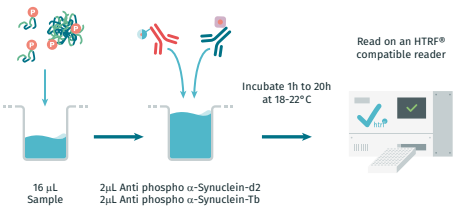


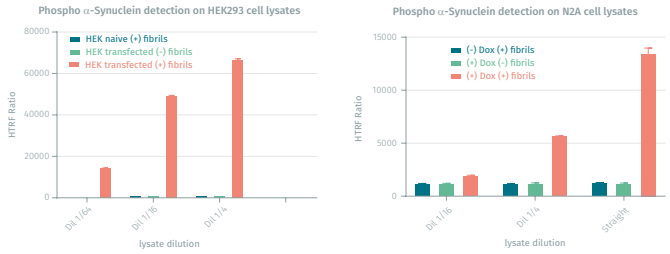
Total α-Synuclein is efficiently quantified in the different dilutions of cell lysates. The HTRF assay displays higher sensitivity and selectivity compared to the BCA detection.

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

Protocol

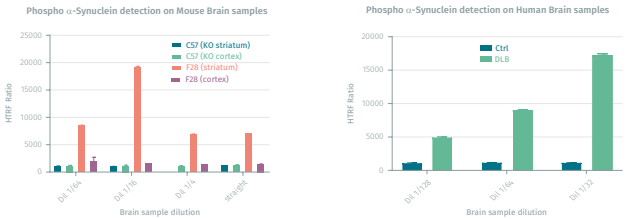
For phospho α-Synuclein (S129) detection, HEK293 over-expressing α-Synuclein and Neuroblastoma N2a cells were seeded with non phosphorylated fibrils, then lysed as recommended in the kit instructions. Following a two-step protocol, phospho α-Synuclein was detected by HTRF as described.





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.



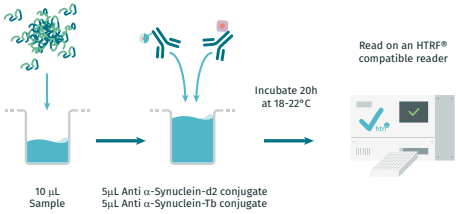
Phosphorylation (Ser 129) could be detected on transgenic mouse (F28) versus control brain samples (C57) and human DLB brain versus control brain samples.

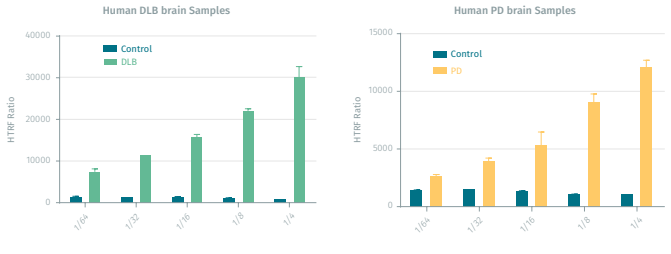
Note that for biological samples, several dilutions must be made to avoid the hook effect.

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.

Europe and other countries +33(0)466 796 705 **U.S. and Canada** 1-888-963-4567 **China** +86 21 5018 9880 **Japan** +81-(0)43-306-8712
www.cisbio.com

ORDERING INFORMATION

INNATE IMMUNITY

Sting Pathway

PRODUCT	500 TESTS	10.000 TESTS
Human STING WT binding kit	64BDSTGPEG	64BDSTGPEH
Human H232 STING binding kit NEW	64BDSTGHPEG	64BDSTGHPEH
Human AQ STING binding kit NEW	64BDSTGQPEG	64BDSTGQPEH
STING phospho-S366 kit	64STGPEG	64STGPEH
STING total kit	64NTGPEG	64NTGPEH
TBK1 phospho-S172 kit	64TBKPEG	64TBKPEH
TBK1 total kit	64NTBPEG	64NTBPEH
IRF3 phospho-S386 kit	6FRF3PEG	6FRF3PEH
NFκB phospho-S536 kit	64NFBPEG	64NFBPEH
NFκB total kit	64NFTPEG	64NFTPEH
Human IFN beta kit	62HIFNBPEG	62HIFNBPEH
Human TNF alpha kit	62HTNFAPEG	62HTNFAPEH

PRODUCT	500 TESTS	10.000 TESTS
Human IL6 kit	62HIL06PEG	62HIL06PEH
Human IL1 beta kit	62HIL1BPEG	62HIL1BPEH

ADAPTIVE IMMUNITY

TCR Pathway

PRODUCT	500 TESTS	10.000 TESTS
ZAP-70 phospho-Y319 kit	64ZAPPEG	64ZAPPEH
ZAP-70 total kit	64ZATPEG	64ZATPEH
SLP-76 phospho S376 kit	63ADK076PEG	63ADK076PEH
SLP-76 total kit	63ADK077PEG	63ADK077PEH
SHP1 phospho-Y564 kit	64SH1PEG	64SH1PEH
SHP1 total kit	64NH1PEG	64NH1PEH
SHP2 phospho-Y542 kit	64SH2PEG	64SH2PEH
SHP2 total kit	64NH2PEG	64NH2PEH
AKT1 phospho-S473 kit	63ADK078PEG	63ADK078PEH
AKT1 total kit	63ADK079PEG	63ADK079PEH
AKT2 phospho S473 kit	63ADK080PEG	63ADK080PEH
AKT2 total kit	63ADK081PEG	63ADK081PEH
AKT3 phospho S473 kit	63ADK082PEG	63ADK082PEH
AKT3 total kit	63ADK083PEG	63ADK083PEH
AKT phospho-S473 kit*	64AKSPEG	64AKSPEH
AKT phospho-T308 kit	64AKTPEG	64AKTPEH
AKT total kit*	64NKTPEG	64NKTPEH
Advanced ERK phospho-T202/Y204 kit*	64AERPEG	64AERPEH
ERK total kit* -	64NRKPEG	64NRKPEH
Human IFN gamma kit*	62HIFNGPEG	62HIFNGPEH
Human IL2 kit	62HIL02PEG	62HIL02PEH

** kits also available under 1 x 96 tests*

Immune Checkpoints

PRODUCT	500 TESTS	10.000 TESTS
PD1/PD-L1 binding assay kit	64ICP01PEG	64ICP01PEH
CD47 / SIRP alpha binding assay kit	64ICP02PEG	64ICP02PEH
LAG3/MHC II binding assay kit	64ICP03PEG	64ICP03PEH
CTLA4/B7-1 binding assay kit	64ICP04PEG	64ICP04PEH
CTLA4/B7-2 binding assay kit	64ICP05PEG	64ICP05PEH

bulk sizes are available

FOR MORE INFORMATION
Europe and other countries +33(0)466 796 705 U.S. and Canada 1-888-963-4567
China +86 21 5018 9880
Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors

PerkinElmer® For the Better | cisbio