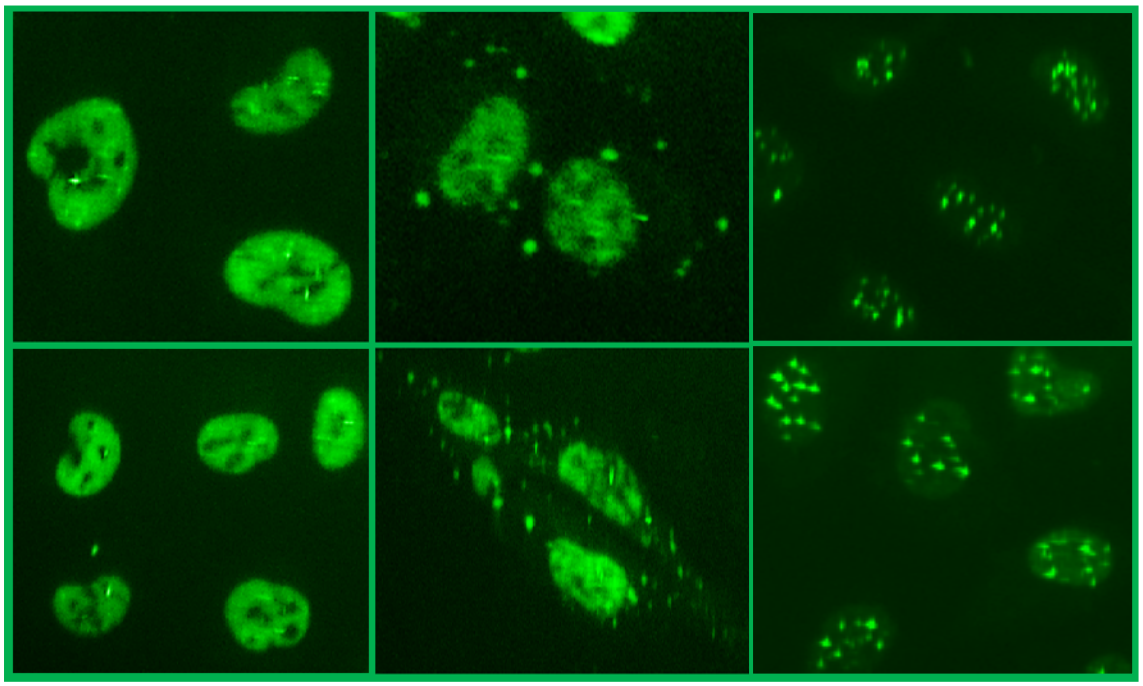
REF: P30710

INNOPROT ASSAYS FOR HIGH CONTENT SCREENING

ALS IN VITRO MODELS

- TDP43 STRESS GRANULES ASSAY CELL LINE -



This cell line has been produced with the technology developed within FP7 PASCA EU project, and is 100% certified truly monoclonal.

Product name: hTDP43-tGFP / U2O2 cell line

Z´: 0.62+/- 0.01

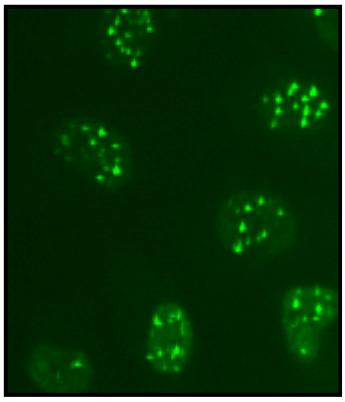
VAT No. ESB95481909

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

REF: P30710

ALS IN VITRO MODEL

TDP-43 STRESS GRANULES ASSAY CELL LINE



Product Name: TDP43-tGFP U2O2 Stable Cell Line

Pathway: TDP-43 globs formation in ALS disease

Official Full Name: TAR DNA-binding protein 43

DNA Accesion Number: GenBank NM_000176

HCS Application: Fluorescent granules quantification

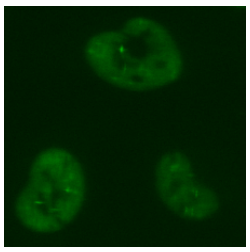
References:

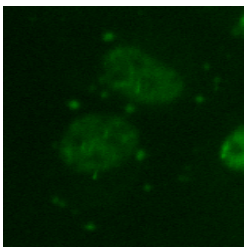
- ***P30710:*** Stable Cell Line (2 vial of cells)
- ***P30710-DA***: Division Arrested cells (2 million cells/vial)

Assay Briefly description

Innoprot's TDP43 Stress Granules Assay cell line has been designed to screen compounds which inhibit the pathological TDP-43 globs formation into the nucleus and cytosol in living cells.

This model also permits to monitor the TDP-43 protein distribution in living cells studying the protein localization pattern in the space and time quantifying the fluorescence aggregation inside the cells.





Each vial of P30710 contains U2OS cells inducible stably expressing human TAR DNA-binding protein 43 (TDP-43) tagged with tGFP.

Background

TAR DNA-binding protein 43, also known ***TDP4,*** is encoded by the ***TARDBP gene***. TARDBP was originally identified as a transcriptional repressor that binds to chromosomally integrated TAR DNA and represses HIV-1 transcription. It was also reported to regulate alternate splicing of the CFTR gene and the apoA-II gene. Later it was discovered that hyper-phosphorylated, ubiquitinated and cleaved form of TARDBP, known as pathologic TDP43, is the major disease protein in ubiquitin-positive, tau-, and alpha-synuclein-negative frontotemporal dementia (FTLD-U now referred to as FTLD-TDP) and in Amyotrophic lateral sclerosis (ALS). Elevated levels of the TDP-43 protein have also been identified in individuals diagnosed with chronic traumatic encephalopathy, a condition that often mimics ALS and that has been associated with athletes who have experienced multiple concussions and other types of head injury.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edf. 502, 1ª Planta | 48160 - Derio
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

REF: P30710

Trafficking of hTDP43-tGFP

In the absence of an oxidative insult, TDP43 protein is predominantly localized in the nucleus. It have been described that in response to oxidative stress and to environmental insults of different types TDP-43 is capable to assemble into stress granules (SGs), ribonucleoprotein complexes where protein synthesis is temporarily arrested.

In addition, it have been speculated that an altered control of mRNA translation in stressful conditions may trigger motor neuron degeneration at early stages of the disease *(Colombrita C, 2009)*. When TDP43 inducible cell model is insulted with sodium arsenite, the protein TDP43 translocates and accumulates into aggregates in the cell cytoplasm. The fluorescent aggregates of hTDP43 can be detected in live cells using an epifluorescence microscope.

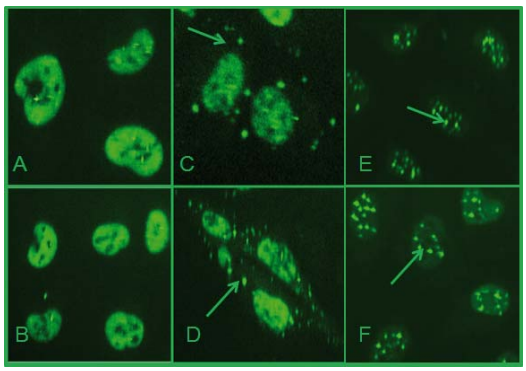


Fig.1. Cellular fluorescence redistribution after sodium arsenite treatment. Representative images of the negative controls show a nuclear distribution of the fluorescence (A,B). However, after sodium arsenite treatment the phenotype turns into a cytosolic vesicular pattern corresponding to stress granules (C,D) and into an intensive nuclear globs pattern (E,F)..

Stress Granules Induction

U2O2 cells stably expressing human TAR DNA-binding protein 43 (TDP-43) were induced with IPTG 5mM during 48h to produce the hTDP43-tGFP protein Subsequently, the cellular model was stimulated with different concentrations of sodium arsenite during 90min. After that, the TDP43 aggregates were detected by fluorescence using image analysis algorithms.

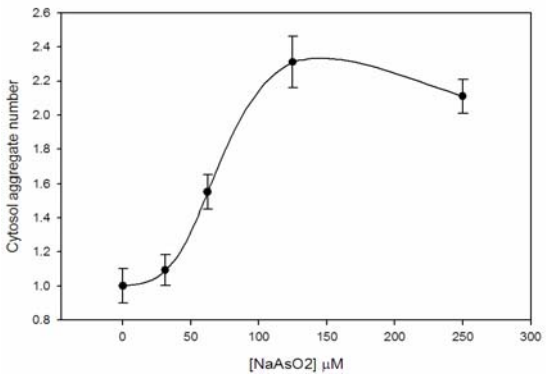


Fig.2. Stress granules appearance after sodium arsenite treatment. Cellular model was treated with 5 mM of IPTG during 72 h to induce the TDP43-tGFP expression. After that, the cells were treated with a range of sodium arsenite concentrations from 25 to 300 uM during 90 min. TDP43 containig stress granules was quantified using the BD Pathway HCS Reader and Attovision Compartimentalization Software. Error bars represent the standard deviation among 3 replicate wells.

Z'= 0.62+/- 0.01 for High Content Screening.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edf. 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

Assay Validation

After TDP43-tGFP expression induction, the cells were incubated with Arimoclomol at 10 uM during 24 hours. Then, the cells were treated with 250 uM sodium arsenite during 90 min. The TDP43-tGFP nuclear globs were quantified using the BD Pathway HCS Reader and Attovision Compartimentalization Software. Error bars represent the standard deviation among 3 replicate wells.

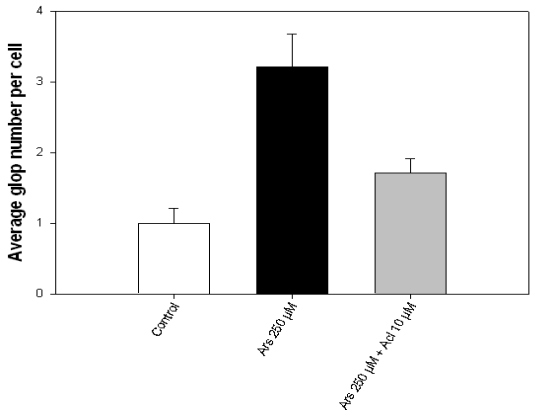


Fig.3. Protective effect of Aromoclimol against oxidative stress.

Applications

The stably transfected TDP43 cell line can be used in drug discovery for pathological globs formation inhibitors.

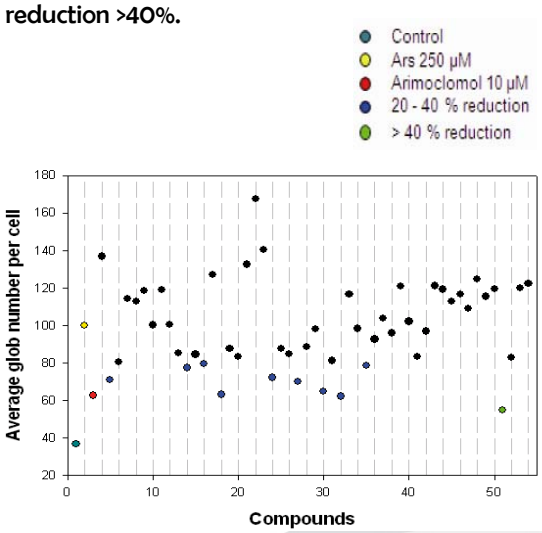
This model permits to evaluate the TDP-43 protein distribution in living cells studying the protein localization pattern.

This cellular model have been adapted to HCS analyses based on image algorithms to test cytosolic and nuclear globs generation process.

Screening Campaign

Before oxidative stress induction by sodium arsenite, the cells were incubated with the compounds at 10 mM during 24 hours. Then the nuclear glob number was quantified using Attovision software. The control of TDP43-tGFP expressing cells is represented in green.

The positive control (Arimoclomol) is represented in red and the negative control (Sodium arsenite) is represented in yellow. The blue spots represent compounds that show a nuclear globs numbers reduction around 20-40%. The light -green spots represent compounds that show a nuclear globs numbers reduction >40%.



Use Restriction

VAT No. ESB95481909
INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edificio 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com