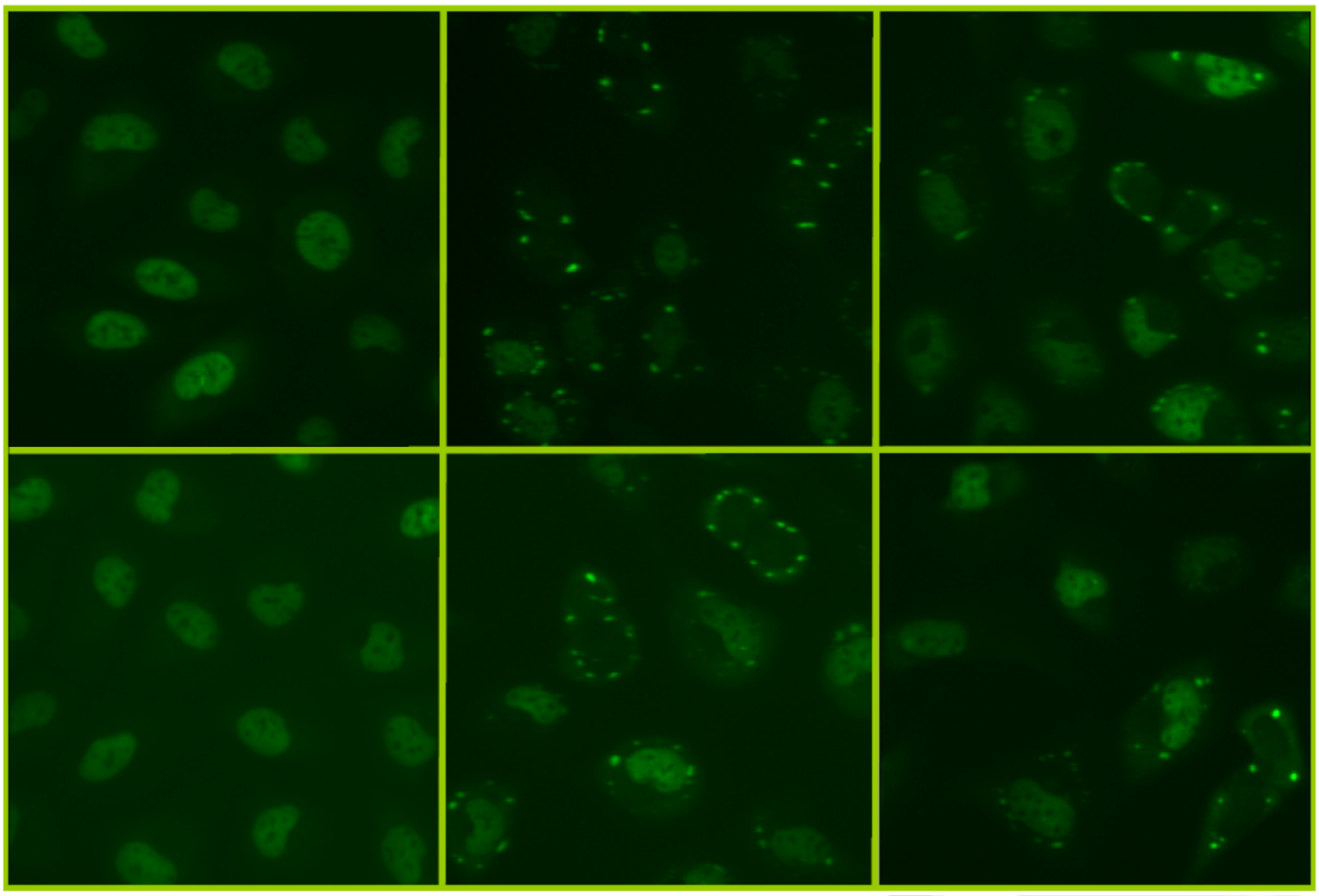
REF: P30716

INNOPROT ASSAYS FOR HIGH CONTENT SCREENING

ALS DISEASE IN VITRO MODELS

- FUS/TLS STRESS GRANULES ASSAY CELL LINE -



Product name: FUS/TLS-tGFP / U2OS cell line

Z´ Arimoclomol / Riluzole: 0.62 +/-0.01

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ALS DISEASE *IN VITRO* MODELS

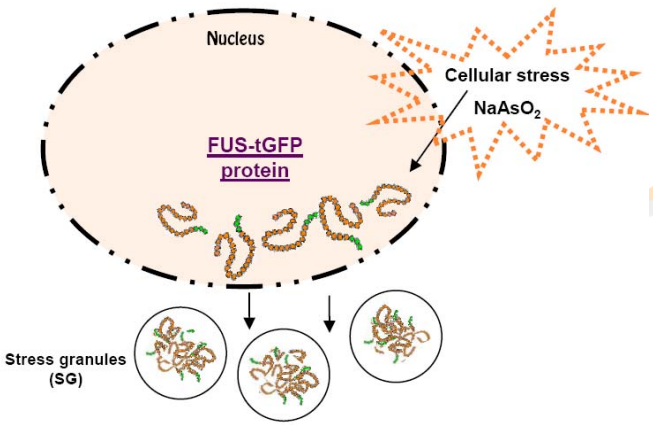
- FUS/TLS STRESS GRANULES ASSAY -

Cell Line Name:	FUS/TLS-tGFP U2OS Stable Cell line
Pathway:	FUS stress granules formation in ALS disease
HCS Application:	Fluorescent granules quantification
Material provided:	P30716: Stable Cell Line (2 vial of cells)
	P30716-DA: Division Arrested cells (2 million cells per vial)

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

Background

ALS is a neurodegenerative disorder that is characterized by premature degeneration of motor neurons, resulting in a progressive, fatal paralysis. The disease is characterized by the selective loss of motor neurons in the brain and spinal cord leading to fatal paralysis. Most cases of ALS are sporadic, but 10% are inherited in a dominant manner (familial ALS).



Cell Line Characteristics

FUS/TLS-tGFP U2Os cells allow to perform assays to evaluate the endogenous formation of Stress granules in living cells.

This cell line has been validated wiht two drugs commonly used to treat ALS disease: Arimoclomol and Riluzole.

This model also permits to monitor FUS/TLS protein distribution in living cells studying the protein localization pattern quantifying the fluorescence aggregation inside the cells.

Use Restriction

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FUS/TLS-tGFP trafficking

Stress granules (SGs) are granules of RNA and proteins formed in the cytosol of the cell under stressful conditions.

Disease-linked mutations of FUS increase its propensity to aggregate and to form SGs by preventing nuclear translocation.

In normal conditions, FUS/TLS protein is predominantly localized in the nucleus (Fig1A-1B). In the presence of an oxidative insult like Sodium arsenite (Fig 1C-1D), FUS/TLS increases its cytoplasmic localization and it is accumulated in Stress granules.

Arimclomol and Riluzole are drugs used in the treatment of ALS disease, a pre-treatment with any of these drugs 24 h before the treatment with Sodium arsenite reduces its toxic effect (Fig 1E-1F).

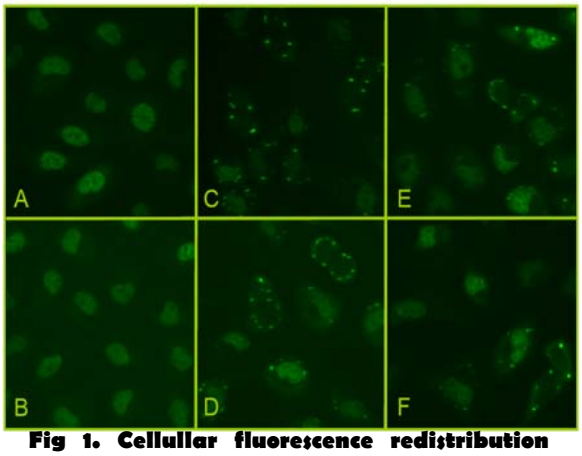

Fig 1. Cellular fluorescence redistribution after an oxidative insult. Figures A-B shows the distribution of FUS/TLS-tGFP protein mostly localized in the nucleus of the cell. After a treatment with Sodium Arsenite 300 µM during 2 h the protein is localized in Stress granules in the cytosol. A pre-treatment of 24 h with Arimoclomol 10 µM (Fig E) reduces aproximately a 12 % the effect of Sodium Arsenite. A pre-treatment of 24 h with Riluzole 10 µM (Fig F) reduces aproximately a 15 % the effect of Sodium Arsenite.

Applications

The stably transfected FUS/TLS-tGFP cell line can be used in drug discovery for pathological stress granules formation inhibitors.

This model permits to evaluate FUS/TLS 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.

Assay Validation

U2OS cells stably expressing FUS/TLS-tGFP construct were treated with Arimoclomol 10 µM or Riluzole 10 µM during 24 h. Afterwards, the cells were treated with Sodium arsenite 300 µM during 2h.

FUS/TLS-tGFP stress granules formation was quantified using the BD Pathway HCS Reader and Attovision Compartimentalization Software. Error bars represent the standard deviation among replicate wells.

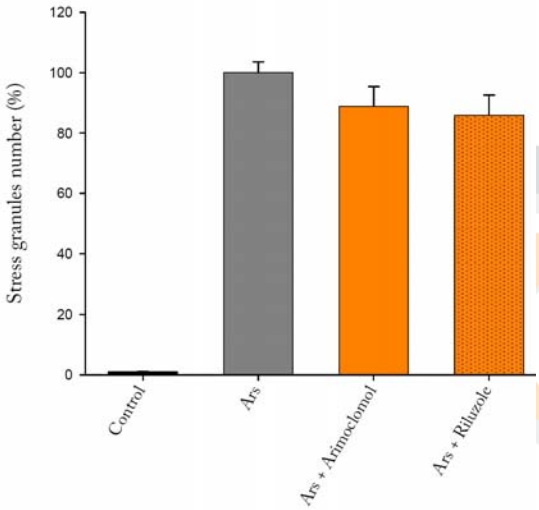

Fig. 2. Protective effect of Arimoclomol and Riluzole against oxidative stress. Arimoclomol pre-treatment reduces Arsenite´s toxicity a 12% and Riluzole a 15%. Z´for this experiment was 0.62 +/- 0.01 and n=12.

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