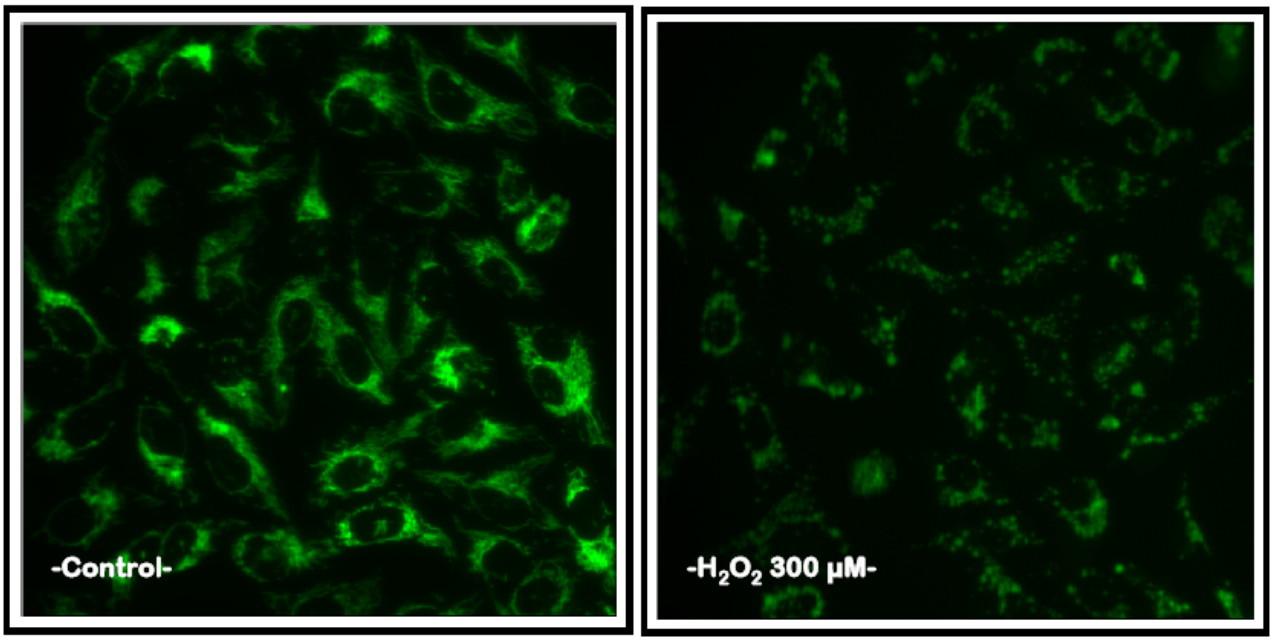
REF: P30805

INNOPROT ASSAYS FOR HIGH CONTENT SCREENING

MITOCHONDRIAL DAMAGE IN VITRO MODEL

- MTS (Mitochondrial targeting sequence)-tGFP U2OS CELL LINE-



Product name: MTS (Mitochondrial targeting sequence)-tGFP U2OS Cell line

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MITOCHONDRIAL DAMAGE *IN VITRO* MODEL

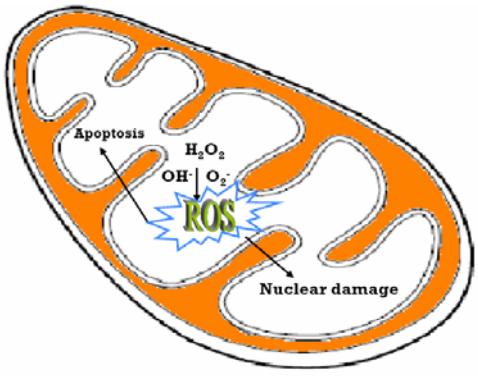
- MTS (Mitochondrial targeting sequence)-tGFP ASSAY -

Cell Line Name:	MTS (Mito. targeting seq.)-tGFP U2OS Cell line
HCS Application:	Mitochondrial damage studies
Material provided:	P30805: Stable Cell Line (2 vials of cells)
	P30805-DA: Division-Arrested cells (2 million cells)

Cell line description

A novel MTS-tGFP U2OS cell line has been developed through stable transfection for monitoring morphological changes in cells during mitochondrial damage.

MTS-tGFP U2OS cell line has been obtained transfecting an expression vector that encodes turbo green fluorescent protein (tGFP) fused to mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase. MTS is fused to the tGFP N-terminus.



Mitochondria, Oxidative stress and Apoptosis

The mitochondria plays an important role in processes related to cellular damage and death like oxidative stress and apoptosis.

Oxidative stress is the consequence of a disparity between the reactive oxygen species produced and the capacity of the system to detoxify the reactive intermediates or to repair the resulting damage. This can cause toxic effects leading into the production of peroxides and free radicals (ROS) that damage the cell .

Apoptosis is the process of programmed cell death (PCD). It is a controlled process in which the cells die but they conserve the molecular components (aminoacids, nucleotides, etc.).

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Assay Validation

U2OS stably expressing MTS-tGFP cells were treated with hydrogen peroxide (H_2O_2) (n=8) during 2 hours. After the treatment it was observed a change in the arrangement of the mitochondria ("balooning").

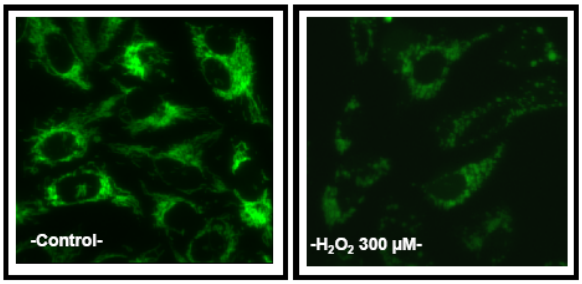

Fig1. H_2O_2 300 µM treatment. Change in the arrangement of the mitochondiria "balooning".

Fluorescence was detected using a BD Pathway 855 High-Content Bioimager. The balooning of the mitochondria was quantified as diferences of fluorescence pattern inside the cell using specific algorithms of Attovision software (BD). When the mitochondria was affected less fluorescence homogeneity was observed. Error bars represent the standard deviation among 8 replicate wells. Z' factor for this experiment was 0,70 +/-0,02.

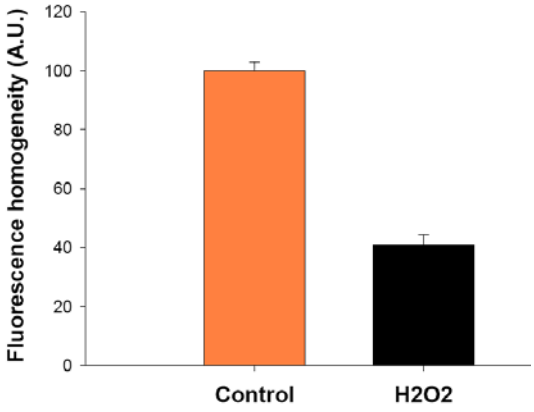

Fig2. Measure of the fluorescence homogeneity before and after **H_2O_2 treatment (300 µM)** . Data represents the mean of 8 replicates and the standar deviation.

Applications

Damage and subsequent dysfunction in mitochondria is an important factor in a range of human diseases due to their influence in cell metabolism.

Oxidative stress is suspected to be important in neurodegenerative diseases such as ALS, Parkinson's disease, Alzheimer's disease, and Huntington's disease . It is thought to be linked to certain cardiovascular disease too.

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