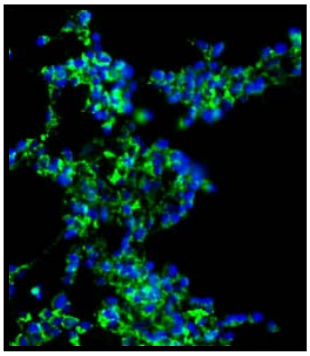
REF: P30801

MITOCHONDRIAL APOPTOSIS ASSAY CELL LINE

Cytc-tGFP HEK293 CELL LINE



Product Name:	Cytc-tGFP / HEK293
Fluorescent Protein:	Cytochrome c
Host Cell:	HEK293
Format:	2 cryopreserved vials
Quantity:	> 3 x 10^6 cells / vial
Storage:	Liquid Nitrogen

Cell Line Briefly description

A novel HEK293/Cytc-tGFP cell line has been developed through stable transfection for monitoring the cellular apoptosis level trhougth the Cytochrome c behavior and morphologicall changes in cell-based assays. HEK293/Cytc-tGFP cell line was obtained by transfection of an expression vector for a fusion protein of mitochondria localization signal from Cytochrome and turboGFP.

Our plasmid was transfected in HEK293 cells, using calcium phosphate method. Cells were then growth with G418 and resistant clones were obtained by limit dilution. Single clones with green fluorescence were selected and allowed to expand. These cells constitutively express the fusion protein Cytc-tGFP

Material Provided

Innoprot provides two vials of stably transfected cryopreserved HEK293 Cells expressing recombinant tGFP tagged cytochrome c.
Each vial contains > 3 x10^6 viable cells post-thawed.

Background

The Cytochrome c is a small protein incorporated in the inner mitochondrial membrane in eucariotic cells. This hemo protein is essential in the electron transport chain for ATP production in cells. The Cytochrome is an intermediate in the apoptosis process. Apoptosis is a controlled form of cell death used to kill cells during their development or in response to an infection or DNA damage. Cytochrome *c* is released by the mitochondria as a consequence of pro-apoptotic stimuli.

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Apoptosis Assay Details

To measure the apoptosis levels, HEK293/Cytc-tGFP were stimulated with staurosporine and nucleus stained with DAPI. In non-apoptotic cells the tGFP appears localizated in mitochondria in perinuclear localization; after treatment tagged mitochondrias appear mislocalizated with a granular shape, and the nucleus stained with DAPI show morphological changes. The assay was developed and optimized using the BD Pathway HCS Reader and Attovision compartimentalization Software.

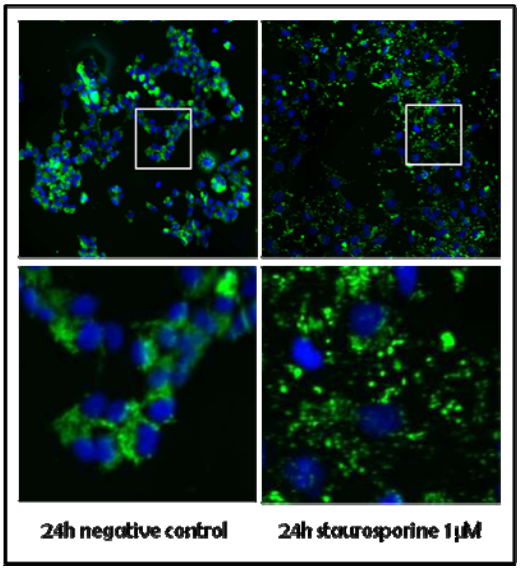

Fig1. DAPI stained cells after treatment without & with staurosporine.

The parameters analyzed in order to check the apoptosis degree are the nucleus shape and the disaggregation of the mitochondria. The last parameter was analyzed as granularity of the tGFP. Both analysis were validated with an average of **Z´=0.61 +/- 0.02 for High Content Screening**.

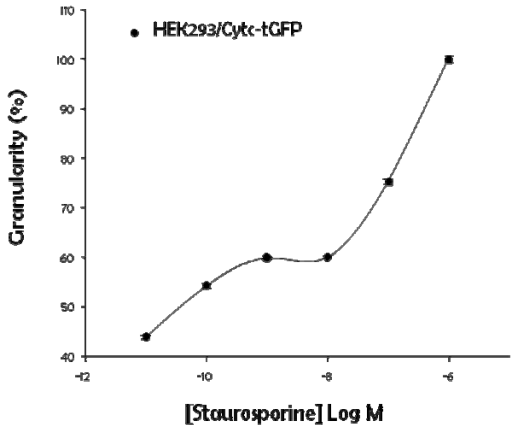

Fig2. Disaggregation of mitochondria assay curve

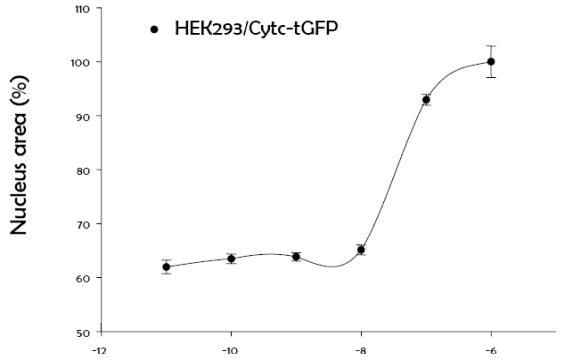

Fig3. Nucleus area assay curve

Applications

- Apoptosis assays
- Mitochondrial Disaggregation studies
- Compartimentalization assays

Use Restriction

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