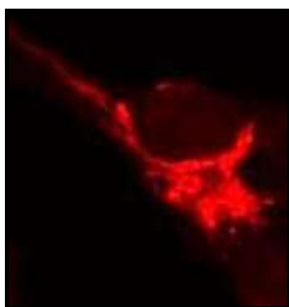


## STABLE CELL LINES FOR APOPTOSIS ASSAYS

### CASPASE 3/7 ACTIVITY ASSAY CELL LINE



<b>Product Name:</b>	MitoCASPred/SH-SY5Y
<b>Host Cell:</b>	SH-SY5Y
<b>Format:</b>	1 cryopreserved vial
<b>Quantity:</b>	> 3 x 10 <sup>6</sup> cells / vial
<b>Storage:</b>	Liquid Nitrogen

#### **Cell Line Briefly Description**

This MitoCASPred/SH-SY5Y cell line has been developed through stable transfection for monitoring the caspase-3 activity level through the turboFP602 protein redistribution in cell-based assays. This cell line was obtained by transfection of an expression vector for a fusion protein of mitochondria localization signal from Cytochrome connected by the linker containing caspase-3 cleavage sequence DEVD and connected by the turboFP602 protein. In living cells the turboFP602 is localized in mitochondria. When the caspase-3 is activated, during early phase of apoptosis, the caspase-3 cleavage the specific DEVD sequence and the FP602 protein shows cellular dispersed localization including nuclear localization.

#### **Material Provided**

Innoprot provides one vial of stably transfected cryopreserved SH-SY5Y/Mitocasp-tFP602. Each vial contains > 3 x 10<sup>6</sup> viable cells post-thawed.

#### **Background**

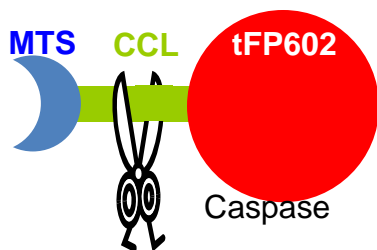
The major executors in the apoptotic program are proteases known as caspases (cysteine-dependent, aspartate-specific proteases). Caspase family orchestrate the changes of the cell during apoptotic process. Caspases exist as precursor, which, when activated initiate the death program by destroying cellular key components and activating factors that mediate damage to the cells. Caspase 3 precursor is activated by cleavage lead to caspase-3 activated form.

#### **About TurboFP602**

TurboFP602 is a red-shifted variant of the red fluorescent protein TurboRFP from sea anemone *Entacmaea quadricolor* [Merzlyak *et al.*, 2007]. TurboFP602 possesses true-red fluorescence (with excitation/emission maxima at 574/602 nm, respectively), optimal for detection via most popular filter sets, and is easily distinguished from background signals. TurboFP602 exhibits fast maturation and high pH stability.

### Assay Details

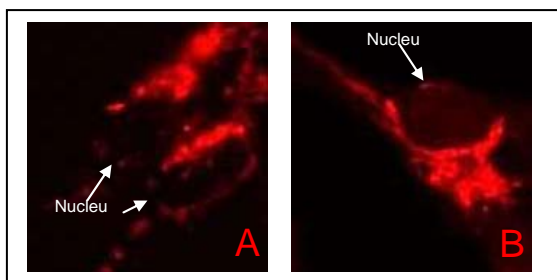
To measure the apoptosis levels, MitoCASPred/SH-SY5Y were stimulated with staurosporine (Fig. 2). In non-apoptotic cells the tFP602 appears localized in mitochondria in perinuclear localization due to de MTS (mitochondrial targeting sequence) situated in the N-terminal of the construct. After promoting apoptosis, casapases are activated and cleave the specific caspase-clavage site (CCS) situated between the MTS and the tFP602 in the construct (Fig. 1), resulting in liberation of tFP602 and entry into the nucleus (Fig. 2).



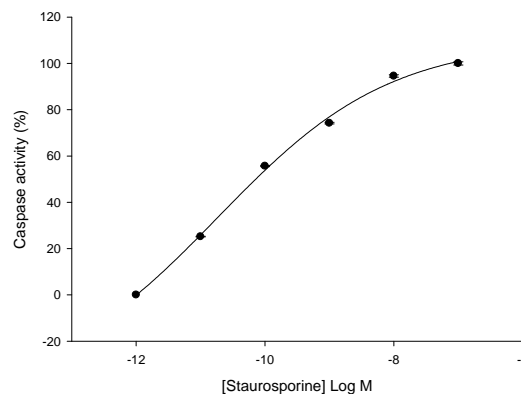
**Fig1. MTS-DEVD-tFP602 and caspase cleavage**

The assay was developed and optimized using the BD Pathway HCS Reader and Attovision compartmentalization Software.

The parameter analyzed in order to check the apoptosis degree is the appearance of red fluorescence (intensity) in the nucleus (Fig. 3).



**Fig2. Mitocasp-tFP602 without (A) and with (B) staurosporine 100 nM.**



**Fig3. Red fluorescence in nuclear area.**

In this curve, the  $EC_{50}$  for staurosporine was 56 pM after a treatment of 24h. Activity was calculated relative to control. This assay was validated with an average of  $Z'$  = **0.62** +/- **0.01** for High Content Screening.

### Quality controls

All cells are performance assayed and test negative for mycoplasma, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation. Innoprot guarantees stable expression for many generations and provides support for cell culture and visualization.

#### Use Restriction

This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein intended to be used for research purposes only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product, or to use the gene or protein other than for non commercial research, including use for validation or screening compounds. For information on commercial licensing, contact Licensing Department, Evrogen JSC, email: [license@evrogen.com](mailto:license@evrogen.com)