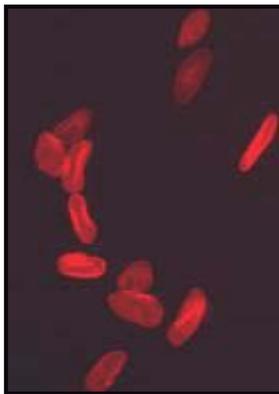


STABLE CELL LINES FOR APOPTOSIS ASSAYS

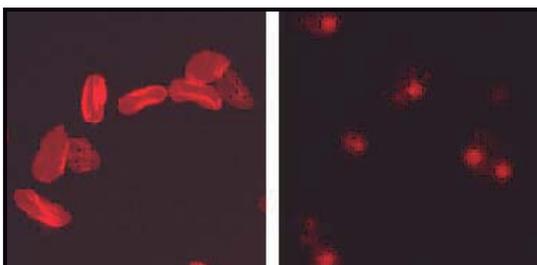
TagRFP-LMNB1/SH-SY5Y CELL LINE



Product Name:	TagRFP-LMNB1/SH-SY5Y
Receptor Name:	Homo sapiens lamin B1
DNA Accession Number:	GenBank NM_005573
Host Cell:	SH-SY5Y
Format:	1 cryopreserved vial
Quantity:	> 3 x 10 ⁶ cells / vial
Storage:	Liquid Nitrogen

Assay Briefly description

Apoptosis is a controlled form of cell death used to kill cells during their development or in response to an infection or DNA damage. During apoptosis, nuclear structure is altered. A SH-SY5Y/TagRFP-LaminB1 cell line has been developed through stable transfection for monitoring the cellular apoptosis level through nuclear lamin morphological changes in cell-based assays.



Each vial of Human tagRFP-laminB1 /SH-SY5Y contains SH-SY5Y cells stably expressing human lamin B1 (LMNB1) tagged in the N-terminus with TagRFP. Innoprot LMNB1 Apoptosis Assay Cell Line has been designed to assay compounds or analyze stimuli for their ability to induce apoptosis in the cells analyzing their nuclear stability.

About Lamin B1

The lamin are type V intermediate filaments which provides structure and mechanical support to the cell nucleus. Besides, they are involved in nuclear stability, reassembly of the nuclear envelope during mitosis, chromatin structure, anchoring of nuclear pore complexes and gene expression. Lamin-B1 is one of the B-type lamins present in humans.

Assay Details

To measure the apoptosis levels, SH-SY5Y/TagRFP-LaminB1 were stimulated with staurosporine. In non-apoptotic cells TagRFP appears localized in nuclear envelope but after treatment, activated caspase family proteases cleave lamin, resulting in the loss of its structure and detachment from chromatine. Tagged lamin appears disassembled and forms round aggregates.

The assay was developed and optimized using the BD Pathway HCS Reader and Attovision Compartmentalization Software. The parameters analyzed in order to check the apoptosis degree are eccentricity and perimeter of the nucleus.

Apoptosis assay based on lamin B1 nuclear morphological changes

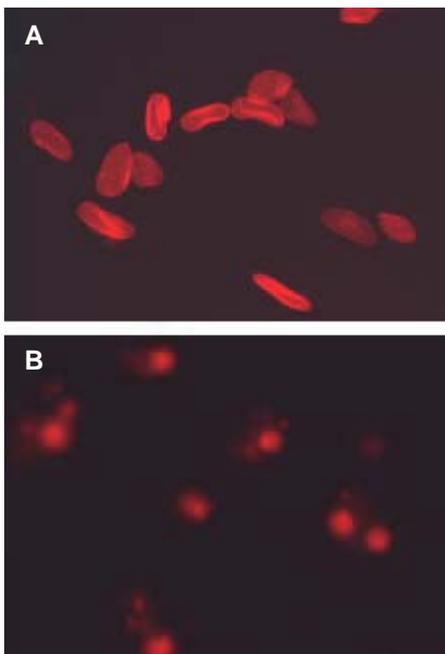


Fig.1. TagRFP-LaminB1 without (A) and with 10 μ M staurosporine.

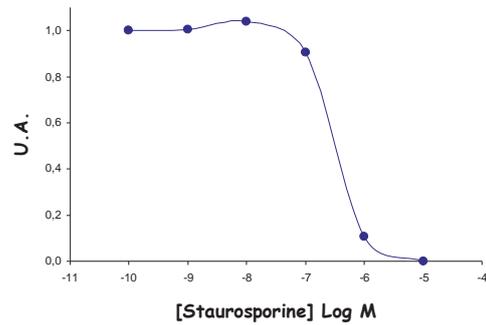


Fig2. Nuclear eccentricity assay curve

In this curve, the EC_{50} for staurosporine was 0.32 μ M after a treatment of 6h. Activity was calculated relative to control. This assay was validated with an average of Z' = 0.72+/- 0.01 for High Content Screening.

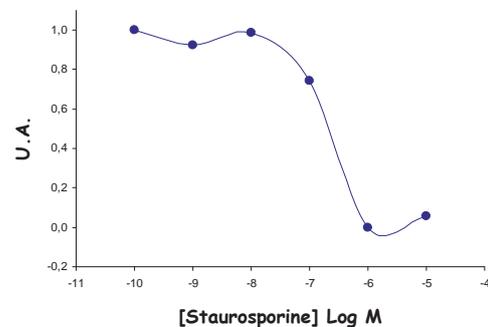


Fig3. Nuclear perimeter assay curve

In this curve, the EC_{50} for staurosporine was 0.13 μ M after a treatment of 6h. Activity was calculated relative to control. This assay was validated with an average of Z' = 0.54+/- 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