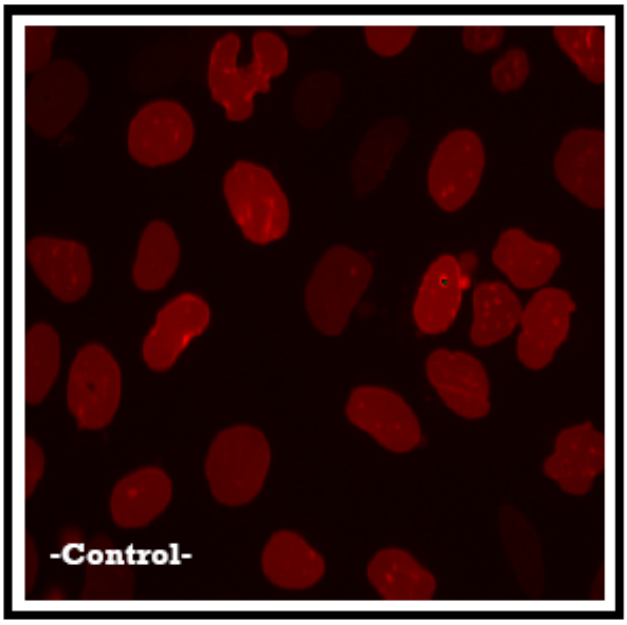
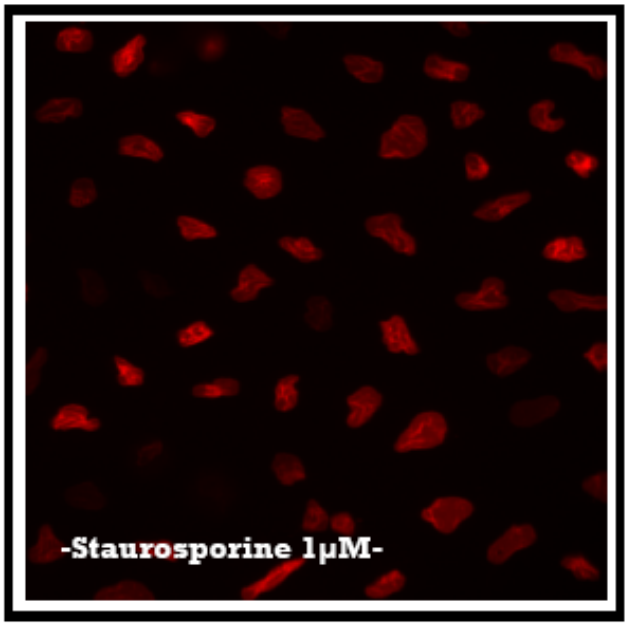
STABLE CELL LINES FOR APOPTOSIS ASSAYS

- Tag-RFP-LAMIN-B1 CELL LINES -





Product name: TagRFP-LaminB1 / U2OS cell line

Ec_{50} Staurosporine: 3.8 x 10^{-8} M

Z´: 0.60+/- 0.02

Product name: TagRFP-LaminB1 / SH-SY5Y cell line

Ec_{50} Staurosporine: 3.2 x 10^{-7} M

Z´: 0.72+/- 0.01

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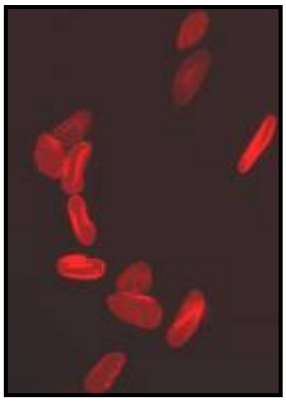
REF: P30601

STABLE CELL LINES FOR APOPTOSIS ASSAYS

TagRFP-LMNB1/SH-SY5Y CELL LINE

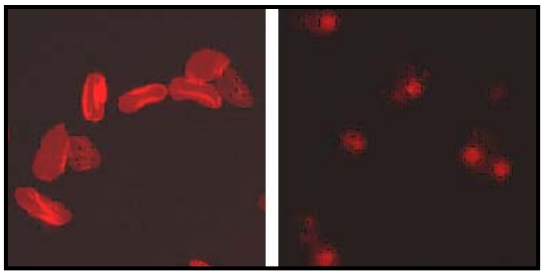


Product Name:	TagRFP-LMNB1/SH-SY5Y
Receptor Name:	Homo sapiens lamin B1
DNA Accesion Number:	GenBank NM_005573
Host Cell:	SH-SY5Y
Format:	1 cryopreserved vial
Quantity:	> 3 x 10^6 cells / vial
Storage:	Liquid Nitrogen

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.

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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 Compartimentalization 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

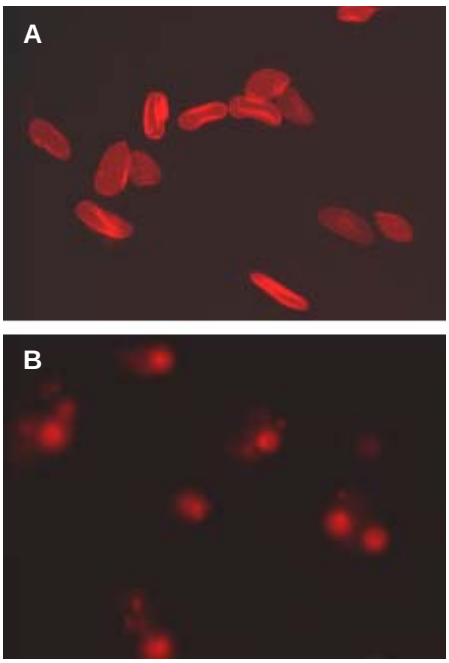

Fig.1. TagRFP-LaminB1 without (A) and with 10 µM (B) staurosporine.

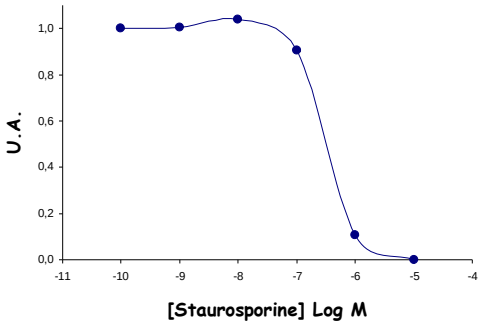

Fig2. Nuclear eccentricity assay curve
In this curve, the Ec50 for staurosporine was 0.32 µM after a treatment of 6h. Activity was calculated relative to control. This assay was validated with an average of **Z'= 0.72+/- 0.01** for High Content Screening.

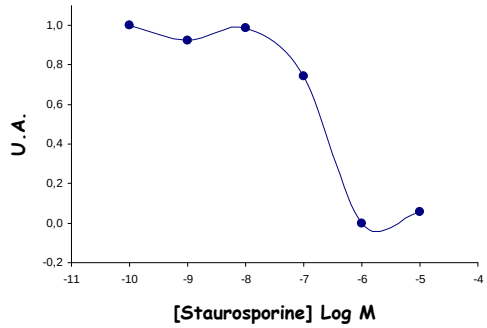

Fig3. Nuclear perimeter assay curve
In this curve, the Ec50 for staurosporine was 0.13 µM after a treatment of 6h. Activity was calculated relative to control. This assay was validated with an average of **Z'= 0.54+/- 0.01** for High Content Screening.

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.

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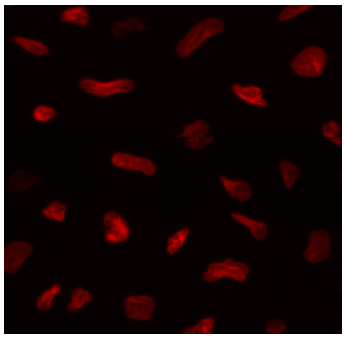
REF: P30807

STABLE CELL LINES FOR APOPTOSIS ASSAYS

TagRFP-LMNB1/U2OS CELL LINE



Products Names: TagRFP-LaminB1/U2OS

Resistance: G418

Format: 1 Cryopreserved vial

Quantity: > 3 x 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

The lamins are type V intermediate filaments which provide 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.

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 U2OS/LaminB1-TagRFP 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/U2OS contains more than 3 million U2OS cells stably expressing human lamin B1 (LMNB1) tagged in the N-terminus with TagRFP. Both Innoprot's LMNB1 Apoptosis Assay Cell Lines has been designed to assay compounds or analyze stimuli for their ability to induce apoptosis in the cells analyzing their nuclear stability

About TagRFP

TagRFP is a monomeric red (orange) fluorescent protein generated from the wild-type RFP from sea anemone *Entacmaea quadricolor* [Merzlyak *et al.*, 2007]. It possesses bright fluorescence with excitation/emission maxima at 555 and 584 nm, respectively. TagRFP is about three times brighter than mCherry protein [Shaner *et al.*, 2004], which makes it the brightest monomeric red fluorescent protein available so far.

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Assay Details (Ec50 = 3.8 x 10^{-8}M)

To measure the apoptosis levels, U2OS/LaminB1-TagRFP were stimulated with Staurosporine.

Tagged lamin appears disassembled and forms round aggregates, loosing its structure.

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

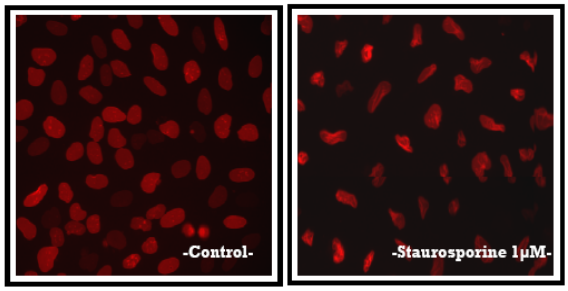

Fig1 Nuclear area assay with Staurosporine.
Concentrations ranging from 0 to 1 µM were tested after 6 h of incubation.

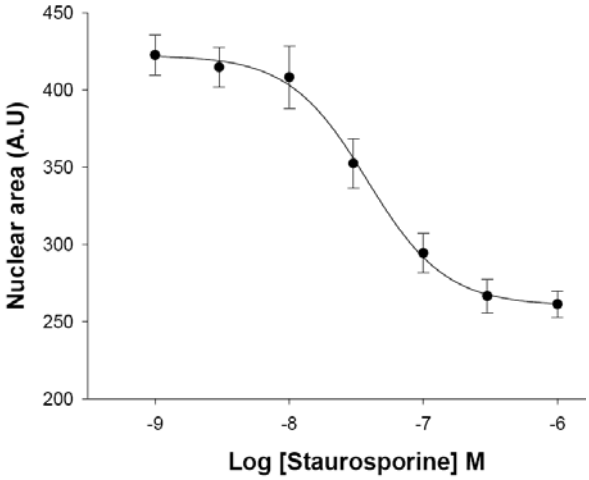

Fig2. Nuclear area assay curve.
Cells were treated with Staurosporine concentrations ranging from 0 to 1 µM, n=6. The EC50 for Staurosporine was ~3.8x10-8M. The apoptosis assay was validated with a Z´= 0.61 +/- 0.02 for High Content Screening.

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.

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