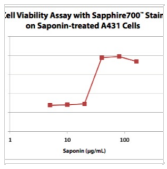

Working

Sep 28, 2018

Cell Viability Assay on Saponin-treated A431 Cells

LI-COR Biosciences[1]

[1]LI-COR Biosciences

dx.doi.org/10.17504/protocols.io.gstbwen

LI-COR Biosciences

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ABSTRACT

This protocol describes a cell viability assay that uses near-infrared fluorescent detection. Sapphire700 Stain is used to determine cell viability by assessing cell membrane integrity, and the assay is imaged with the Odyssey CLx Imaging System.

EXTERNAL LINK

https://www.licor.com/documents/s6xiekspf0z3a802h8gnvina5ayydmyw

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

1. Gani OA, Engh RA (2010) Protein kinase inhibition of clinically important staurosporine analogues. Nat Prod Rep. 27: 489-98

2. Gescher A. (2000) Staurosporine analogues – pharmacological toys or useful antitumor agents? Crit Rev Oncol Hematol. 34: 127-35

3. Venditto VJ, Simanek EE (2010) Cancer therapies utilizing the camptothecins: a review of the in vivo literature. Mol Pharm. 7: 307-49

AppNote_OdyCLxSa_CellViabilitySapphire700_0314_979-14115.pdf

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Introduction

Cell viability can be assessed based on various cellular features and mechanisms. These include cell membrane integrity (detected by cell impermeable dyes or leakage of intracellular lactate dehydrogenase (LDH) activity), monitoring of ATP with bioluminescence assays, determining esterase activity with Calcein-AM or Fluorescein-DA, measuring cellular Redox status with MTT, MTS, WST, or XTT, and detecting the mitochondrial membrane potential with JC-1. Various cell viability assays have been developed for plate readers (monitoring absorbance and luminescence), flow cytometry, and image cytometry (e.g. NucleoCounter® NC-3000TM from ChemoMetec); however, none of these assays have been optimized for near-infrared detection with the Odyssey Imaging System.

This protocol describes a cell viability assay that uses near-infrared fluorescent detection. Sapphire700 Stain is used to determine cell viability by assessing cell membrane integrity, and the assay is imaged with the Odyssey CLx Imaging System.
Sapphire700 Stain is cell impermeable and non-fluorescent in healthy, intact cells.

When the cell membrane is damaged, the stain binds to intracellular proteins and becomes fluorescent.

Fluorescence intensity in the 700 nm channel is correlated to the number of cells with compromised membranes.

Convenient, 'mix-and-read' homogenous assay requires no washing or reagent transfer steps (shown in Figure 1).

This assay measures the total fluorescence in each microplate well, but does not image or count individual cells.

For this technical note, A431, Jurkat, and RAW264.7 cells were evaluated. Other cell lines may require optimization. Cell death was induced by applying different concentrations of Staurosporine (STS), Camptothecin (CPT) or Saponin.

Saponins are natural surfactants or detergents, found in many plants, that are used to permeabilize or lyse cells.

CPT, a cytotoxic quinolone alkaloid extracted from Camptotheca acuminate, is a potent inhibitor of topoisomerase I, an enzyme required for DNA synthesis. CPT induces apoptosis in a dose-dependent manner in vitro and is routinely used as a general method for inducing apoptosis[1,2].

STS is an alkaloid originally isolated from bacterium Streptomyces staurosporeus. STS is an inhibitor of phospholipid/Ca2+ dependent protein kinase (Protein Kinase C; PKC), and prevents binding of ATP to the kinase. There are multiple ways in which STS induces apoptosis. One way is by activating caspase-3. STS is used to induce apoptosis in many mammalian cell types[3].

Figure 1. Workflow for Cell Viability Assay with Sapphire700 Stain on Odyssey Imaging System.

Materials

LI-COR Reagents

Sapphire700 Stain (LI-COR, P/N 928-40022)

Additional Materials

Tissue culture dishes, 100 x 20 mm style (BD Falcon P/N 353003)

96-well plate, flat bottom, tissue culture treated, black wall with clear bottom (Costar, P/N 3904)

RAW264.7 cells (ATCC® TIB-71TM)

A431 cells (ATCC® CRL-1555TM)

Jurkat cells, Clone E6-1 (ATCC® TIB-152TM)

Fetal Bovine Serum (FBS) (ATCC, P/N 30-2020)

Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, P/N D5796)

RPMI-1640 (Sigma, P/N R5886)

0.05% Trypsin-EDTA (1X) (GIBCO, P/N 25300-054)

Saponin (Sigma, P/N S4521)

Staurosporine (Sigma, P/N S5921)

Camptothecin (Sigma, P/N C9911)

Result

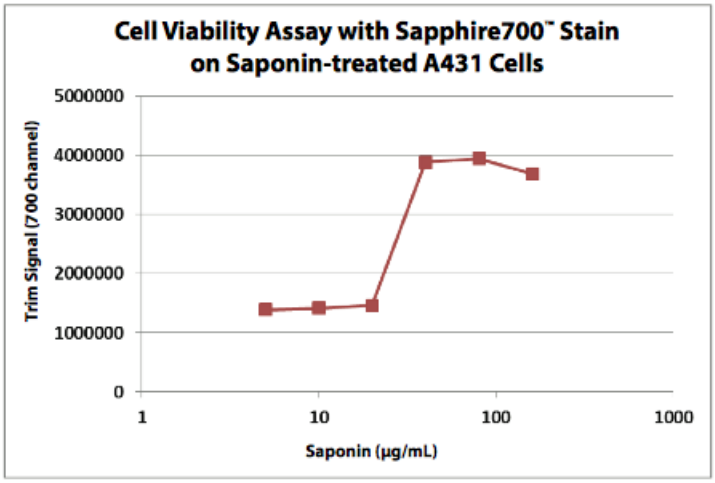

Figure 2. Cell Viability Assay with Sapphire700 Stain on Saponin-treated A431 Cells.

A431 cells (~ 40,000 cells/well) were grown in a 96-well plate. Cells were treated with Saponin at concentrations ranging from 5 to 160 µg/mL for 1 hour and cell viability was assessed with Sapphire700 Stain (1:100). The plate was scanned with an Odyssey CLx Imager (resolution: 169 µm; quality: medium; focus offset: 4.0 mm; intensity: 5). The Trim Signals of the 700 nm channel were used to generate the graph.

MATERIALS

NAME	CATALOG #	VENDOR
Sapphire700™ Stain	P/N 928-40022	LI-COR
75-cm2 cell culture flask	P/N 430641	Corning
96-well plate, flat bottom, tissue culture treated, black wall with clear bottom	3904	Fisher Scientific
A431 cells	CRL-1555	ATCC
Fetal Bovine Serum (FBS)	30-2020	ATCC
Dulbecco's Modified Eagle's Medium (DMEM)	D5796	Sigma Aldrich
RPMI-1640	R5886	Sigma Aldrich
0.05% Trypsin-EDTA (1X)	25300-054	Thermo Fisher Scientific
Saponin	S4521	Sigma Aldrich

SAFETY WARNINGS

See SDS for safety and warnings.

Cell Preparation

1 Grow A431 cells in a 100-mm tissue culture dish with growth medium (DMEM supplemented with 10% FBS) using standard cell culture practices. Always make sure that cells are healthy before using them for the experiment.

Saponin Treatment

2 The day before the experiment, dislodge cells from the dish with trypsin and suspend cells in 10 mL of growth medium; count cells, then seed directly into a 96-well plate at a volume of
200 µL containing 4 x 10^4 cells per well.

NOTE

IMPORTANT: Cells MUST be healthy and not overcrowded. The outcome of this experiment will be significantly affected by the condition of the cells.

10 ml **Growth medium**

200 µl **seeding volume**

3 Grow cells overnight in a humidified CO_2 cell culture incubator at 37 °C.

18:00:00

37 °C **incubation temperature**

4 The next day, replace the medium with 50 µL of DMEM containing saponin (5 to 160 µg/mL) without FBS.

50 µl DMEM containing saponin w/o FBS

5 Incubate cells for 60 minutes in a humidified CO_2 cell culture incubator at 37 °C. Leave cells untreated as the negative control.

01:00:00

37 °C incubation

Sapphire700 Staining

6 Add 50 µL of Sapphire700 Stain (1:50 dilution in DMEM) to each well.

50 µl Sapphire700 Stain

7 Incubate cells in a humidified CO_2 cell culture incubator at 37 °C for 30 minutes.

00:30:00

37 °C incubation

8 Scan the plate with detection in the 700 nm channel, using an Odyssey CLx Imager.