Technical Note

Cell Viability Assay with Sapphire700™ Stain and the Odyssey® CLx and Sa Imaging Systems

Developed for:

Aerius, Odyssey Classic, Odyssey CLx, and Odyssey Sa Imaging Systems

Please refer to your manual to confirm that this protocol is appropriate for the applications compatible with your Odyssey Imager model.

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I. 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 mitochondria 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-3000™ 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/Ca^{2+} 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].

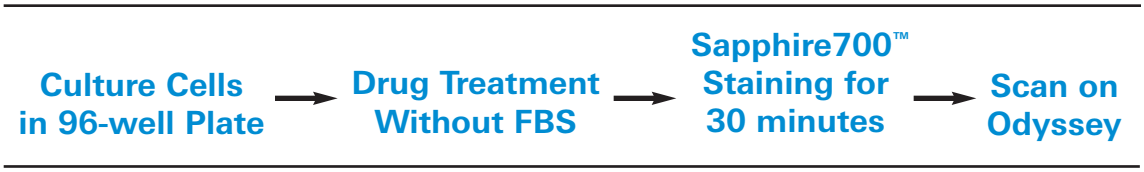

Figure 1. Workflow for Cell Viability Assay with Sapphire700 Stain on Odyssey Imaging System.

II. 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)
- 75-cm^2 cell culture flask (Corning P/N 430641)
- 96-well plate, flat bottom, tissue culture treated, black wall with clear bottom (Costar, P/N 3904)
- RAW264.7 cells (ATCC® TIB-71™)
- A431 cells (ATCC® CRL-1555™)
- Jurkat cells, Clone E6-1 (ATCC® TIB-152™)
- 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)

III. METHODS, PROCEDURES, AND RESULTS

a. Cell Viability Assay on Saponin-treated A431 Cells

Cell Preparation

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

1. 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 (Costar, P/N 3904) at a volume of 200 µL containing 4 x 10^4 cells per well. Grow cells overnight in a humidified CO_2 cell culture incubator at 37 °C.

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

2. The next day, replace the medium with 50 µL of DMEM containing saponin (5 to 160 µg/mL) without FBS and incubate cells for 60 minutes in a humidified CO_2 cell culture incubator at 37 °C. Leave cells untreated as the negative control.

Sapphire700 Staining

1. Add 50 µL of Sapphire700 Stain (1:50 dilution in DMEM) to each well and incubate cells in a humidified CO_2 cell culture incubator at 37 °C for 30 minutes.

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

Result

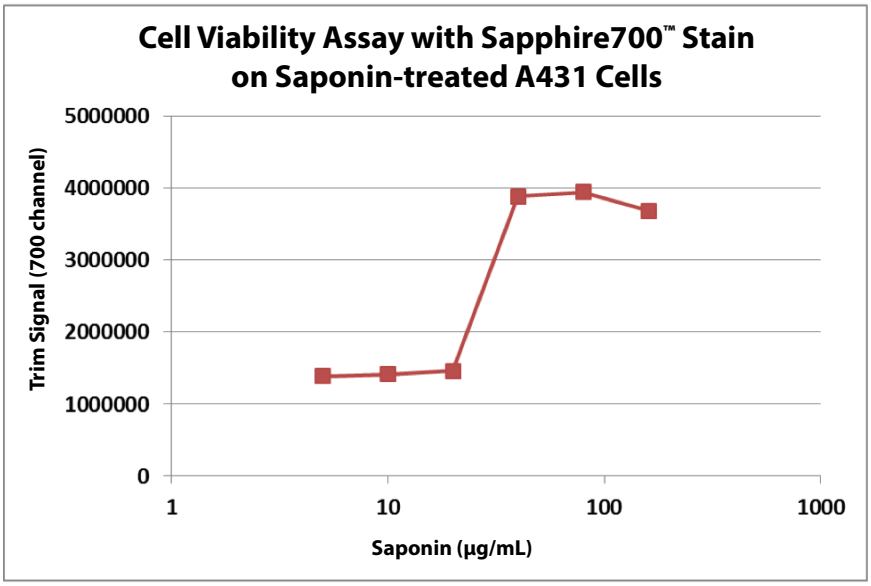

Figure 2. Cell Viability Assay with Sapphire700 Stain on Saponin-treated A431 Cells.

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.

b. Cell Viability Assay with Staurosporine-treated Jurkat Cells

Cell Preparation

Grow Jurkat cells in a 75-cm^2 cell culture flask with growth medium (RPIM-1640 supplemented with 10% FBS) using standard cell culture practices. Always make sure that cells are healthy before using them for the experiment.

Staurosporine Treatment

The day before the experiment, disperse cells in 10 mL of growth medium by pipetting in and out several times; count cells, then re-suspend cells with RPMI-1640 containing Staurosporine (0.625 to 320 µM) without FBS. Seed cells into a 96-well plate (Costar, P/N 3904) in a volume of 50 µL containing 5 x 10^4 cells per well. Grow cells overnight in a humidified CO_2 cell culture incubator at 37 °C. Use the same number of cells without Staurosporine treatment as control.

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

Sapphire700 Staining

1. Add 50 µL of Sapphire700 Stain (1:50 dilution in RPMI-1640) to each well and incubate cells in a humidified CO_2 cell culture incubator at 37 °C for 30 minutes.

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

Result

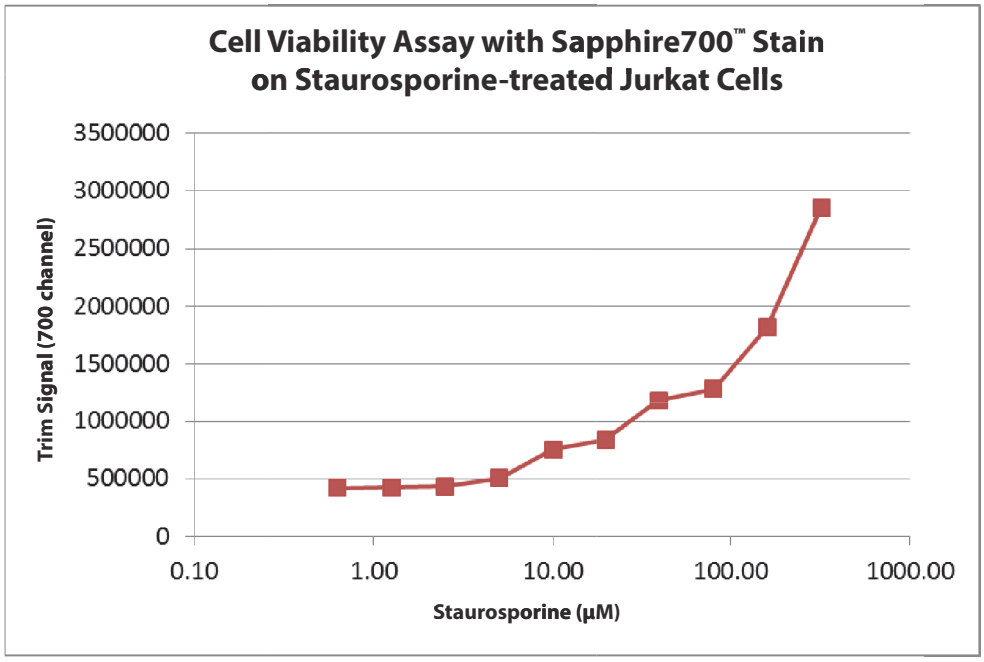

Figure 3. Cell Viability Assay with Sapphire700 Stain on Staurosporine-treated Jurkat Cells. Jurkat cells (~ 50,000 cells/well) were grown in a 96-well plate. Cells were treated with Staurosporine at concentrations ranging from 0.625 to 320 µM for 28 hours and cell viability was assessed with Sapphire700 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.

c. Cell Viability Assay with Camptothecin-treated RAW264.7 Cells

Cell Preparation

Grow RAW264.7 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.

Camptothecin Treatment

The day before the experiment, scrape cells into 5 mL of DMEM without FBS and disperse cells by pipetting in and out several times; count cells, then seed cells directly into a 96-well plate (Costar, P/N 3904) at a volume of 50 µL containing 5 x 10^4 cells per well in the presence of Camptothecin (1.25 to 160 µM). Grow cells overnight in a humidified CO_2 cell culture incubator at 37 °C. Use the same number of cells without Camptothecin treatment as control.

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

Sapphire700 Staining

3. Add 50 µL of Sapphire700 Stain (1:50 dilution in DMEM) to each well and incubate cells in a humidified CO_2 cell culture incubator at 37 °C for 30 minutes.

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

Result

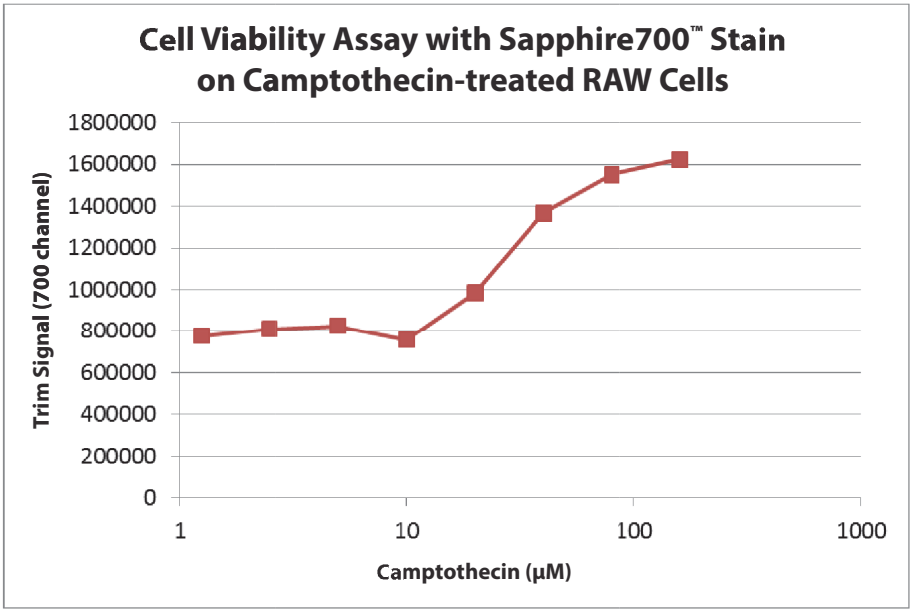

Figure 4. Cell Viability Assay with Sapphire700 Stain on Camptothecin-treated RAW264.7 Cells. RAW 264.7 cells (~ 50,000 cells/well) were grown in a 96-well plate. Cells were treated with Camptothecin at concentrations ranging from 1.25 to 160 µM for 20 hours and cell viability was assessed with Sapphire700 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.

IV. REFERENCES

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

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