Cell Meter™ Nuclear Apoptosis Assay Kit *Green Fluorescence Optimized for Flow Cytometry*

Catalog number: 22811
Unit size: 100 Tests

Component	Storage	Amount
Component A: 200X Nuclear Green™ DCS1	Freeze (<-15 °C), Minimize light exposure	1 vial (500 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (100 mL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used for monitoring cell viability. This particular kit is designed to monitor cell apoptosis through measuring the apoptotic chromatin condensation. The compacted chromatin of apoptotic cells binds higher amounts of nuclear dye compared to the healthy cells. Our Cell Meter™ Nuclear Apoptosis Assay Kit provides all the essential components with an optimized assay method for the detection of apoptosis in cells with condensed nuclei. This fluorometric assay is based on the detection of the DNA contents in cells using our proprietary non-fluorescent dye that becomes strongly fluorescent upon binding to DNA. In normal cells, Nuclear Green™ is not cell permeable, however, in apoptotic cells, Cells with compromised plasma membranes or with impaired/no cell metabolism are unable to prevent the dye from entering the cell. Once inside the cell, the dyes bind to intracellular DNA producing highly fluorescent complexes which identify the cells as non-viable cells. The staining with Nuclear Green; DCS1 can be measured using a flow cytometer (FL1 channel) or fluorescence microscope (FITC filter set). The kit can be used with our other apoptosis reagents, such as Our Cell Meter™ NIR Mitochondria Membrane Potential Detection Kit (Cat# 22802), for multi-parametric study of cell viability and apoptosis. The kit is optimized for screening of apoptosis activators and inhibitors.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds at a density of 5×10^5 to 1×10^6 cells/mL
2. Add 5 µL of 200X Nuclear Green™ DCS1 into 1 mL of cell solution
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 to 60 minutes
4. Pellet the cells and resuspend the cells in 1 mL of growth medium
5. Analyze the fluorescence intensity using flow cytometer with FL1 channel

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm filter
Instrument specification(s):	FITC channel

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. For each sample, prepare cells in 1 mL of warm medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.

   Note Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

2. Treat cells with test compounds for a desired period of time to induce apoptosis.

3. Add 5 µL of 200X Nuclear Green™ DCS1 (Component A) into the treated cells.

4. Incubate the cell solution in a 37°C, 5% CO_2 incubator for 30 to 60 minutes.

   Note For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intack, and wash the cells once with serum-containing media prior to the incubation with Nuclear Green™ DCS1 dye-loading solution. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

5. **Optional:** Centrifuge the cells at 1000 rpm for 4 minutes, and then re-suspend cells in 1 mL of Assay Buffer (Component B) or buffer of your choice.

6. Monitor the fluorescence intensity using a flow cytometer with FL1 channel (Ex/Em = 490/525 nm). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

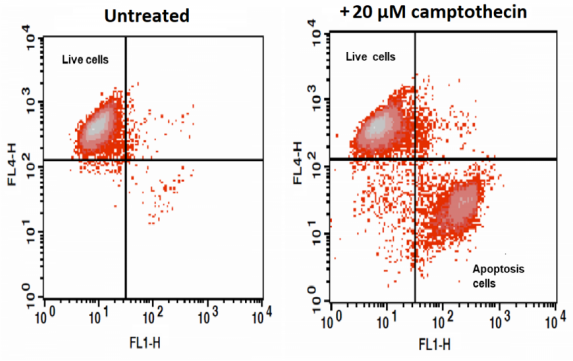

Figure 1. The increase in fluorescence intensity of Nuclear Green™ DCS1 with the addition of Camptothecin in Jurkat cells. Jurkat cells were treated overnight without (Left) or with 20 µM camptothecin (Right) in a 37 oC, 5% CO2 incubator, and then dye loaded with Nuclear Green™ DCS1 for 60 minutes. At the end of 15 minutes of Nuclear Green™ DCS1 dye loading, MitoLite™ NIR (Cat. # 22802) was added for multicolor analysis. The fluorescence intensity of Nuclear Green™ DCS1 and MitoLite™ NIR was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using FL1 channel (Nuclear Green™ DCS1) and FL4 channel (MitoLite™ NIR).

DISCLAIMER