Cell Meter™ Nuclear Apoptosis Assay Kit

Green Fluorescence, Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22811 (100 assays)	Keep in freezer and avoid exposure to light	Flow Cytometer

Introduction

This particular kit is designed to monitor cell apoptosis by measuring the apoptotic chromatin condensation. The compacted chromatin of apoptotic cells binds higher amounts of nuclear dye compared to the healthy cells. The 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 cellular DNA.

In normal cells, Nuclear Green™ DCS1 is not cell permeable. However, in apoptotic cells, Nuclear Green™ DCS1 can easily get into the cells with compromised plasma membranes. Once inside the cell, the dye binds to intracellular DNA producing highly fluorescent complexes which identify the cells as non-viable cells. The staining density with Nuclear Green™ DCS1 can be measured with a flow cytometer at Ex/Em = 490/520 nm (FL1 channel) or a fluorescence microscope (FITC filter set). The kit can be used together with our other apoptosis reagents, such as our Cell Meter™ NIR Mitochondrial Membrane Potential Detection Kit (#22802), for multi-parametric study of cell viability and apoptosis. The kit is optimized for screening apoptosis activators and inhibitors.

Kit Components

Components	**Amount**
Component A: 200X Nuclear Green™ DCS1	1 vial (500 µL)
Component B: Assay Buffer	1 bottle (100 mL)

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells with test compounds at a density of 5×10^5 to 1×10^6 cells/mL → Add 5 µL of 200X Nuclear Green™ DCS1 into 1 mL of cell solution → Incubate at room temperature for 30-60 minutes → Pellet the cells and resuspend the cells in 1 mL of growth medium → Analyze the fluorescence intensity with a flow cytometer

Note: Thaw all the kit components at room temperature before use.

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 (from Step 2), and incubate the cell solution in a 37 °C, 5% CO_2 incubator for 30 to 60 minutes.

   Note1: 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.

   Note 2: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

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Technical Support: support@aatbio.com; Tel: 408-733-1055

4. *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.

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

Data Analysis

In live non-apoptotic cells, non-permeable Nuclear Green™ DCS1 can't stain the nuclea. However, the staining intensity is increased when the Nuclear Green™ DCS1 gets into the apoptotic cells and binds to compacted chromatin.

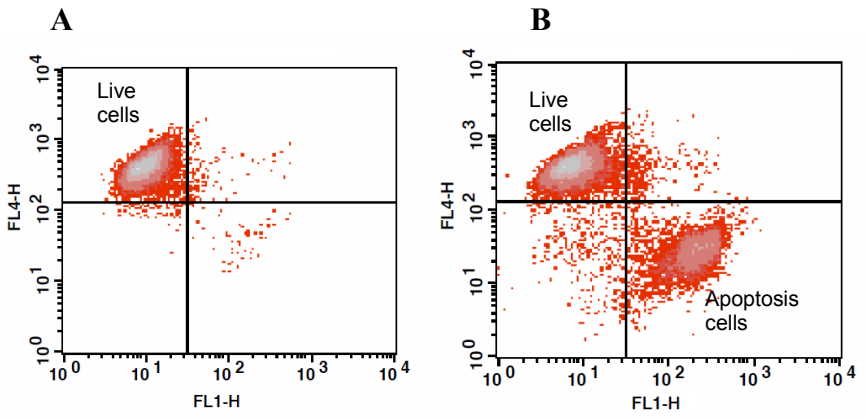

Figure 1. The increase in fluorescence intensity of Nuclear Green™ DCS1 with the addition of Camptothecin in Jurkat cells. Jurkat cells were treated overnight without (A) or with 20 µM camptothecin (B) in a 37 ^{o}C, 5% CO_2 incubator, and then dye loaded with Nuclear Green™ DCS1 for 60 minutes. At the 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).

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.