Cell Meter™ Fluorimetric Cell Cycle Assay Kit

Green Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22841 (100 assays)	Keep in freezer and avoid exposure to light	Flow Cytometer

Introduction

The cell cycle has four sequential phases: G0/G1, S, G2, and M. During a cell's passage through cell cycle, its DNA is duplicated in S (synthesis) phase and distributed equally between two daughter cells in M (mitosis) phase. These two phases are separated by two gap phases: G0/G1 and G2. The two gap phases provide time for the cell to grow and double the mass of their proteins and organelles. They are also used by the cells to monitor internal and external conditions before proceeding with the next phase of cell cycle. The cell's passage through cell cycle is controlled by a host of different regulatory proteins.

This particular kit is designed to monitor cell cycle progression and proliferation by using our proprietary Nuclear Green™ LCS1 in permeabilized and fixed cells. The percentage of cells in a given sample that are in G0/G1, S and G2/M phases, as well as the cells in the sub-G1 phase prior to apoptosis can be determined by flow cytometry. Cells stained with Nuclear Green™ LCS1 can be monitored with a flow cytometer at Ex/Em = 490 nm/520 nm (FL1 channel).

Kit Components

Components	**Amount**
Component A: 200X Nuclear Green™ CCS1	1 vial (250 µL)
Component B: Assay Buffer	1 bottle (50 mL)

Assay Protocol

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™ CCS1 into 0.5 mL of cell solution → Incubate at room temperature for 30-60 minutes → Analyze with a flow cytometer using the FL1 channel

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

1. For each sample, prepare cells in 0.5 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 or other cell cycle functions.
3. Add 2.5 µL of 200X Nuclear Green™ CCS1 (Component A), and incubate the cells in a 37 °C, 5% CO_2 incubator for 30 to 60 minutes.
   Note 1: For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with Nuclear Green™ CCS1.
   Note 2: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
   Note 3: It is not necessary to fix the cells before DNA staining since the Nuclear Green™ CCS1 is cell-permeable.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
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 0.5 mL of assay buffer (Component B) or the buffer of your choice.

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

Data Analysis

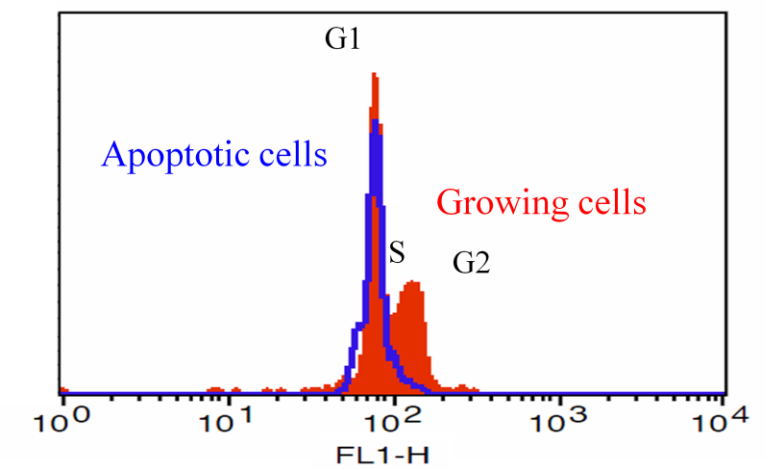

Figure 1. DNA profile in growing and camptothecin treated Jurkat cells. Jurkat cells were treated without (red) or with 20 µM camptothecin (blue) in a 37 °C, 5% CO_2 incubator for about 8 hours, and then dye loaded with Nuclear Green™ CCS1 for 60 minutes. The fluorescence intensity of Nuclear Green™ CCS1 was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using the FL1 channel. In growing Jurkat cells, nuclear stained with Nuclear Green™ CCS1 shows G1, S, and G2 phases (red). In camptothecin treated apoptotic cells (B), the fluorescence intensity of Nuclear Green™ CCS1was decreased, and both S and G2 phases were diminished.