Cell Meter™ Fluorimetric Live Cell Cycle Assay Kit *Green Fluorescence Optimized for Flow Cytometry*

Catalog number: 22841
Unit size: 100 Tests

Component	Storage	Amount
Component A: 200X Nuclear Green™ LCS1	Freeze (< -15 °C), Minimize light exposure	1 vial (250 µL)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (50 mL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability and proliferation. There are a variety of parameters that can be used for monitoring cell viability and proliferation. In normal cells, DNA density changes depending on whether the cell is growing, dividing, resting, or performing its ordinary functions. The progression of the cell cycle is controlled by a complex interplay among various cell cycle regulators. These regulators activate transcription factors, which bind to DNA and turn on or off the production of proteins that result in cell division. Any misstep in this regulatory cascade causes abnormal cell proliferation which underlies many pathological conditions, such as tumor formation. Potential applications for live-cell studies are in the determination of cellular DNA content and cell cycle distribution for the detection of variations in growth patterns, for monitoring apoptosis, and for evaluating tumor cell behavior and suppressor gene mechanisms. This particular kit is designed to monitor cell cycle progression and proliferation using our proprietary Nuclear Green™ LCS1 in live, 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 (FL1 channel).

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 2.5 µL of 200X Nuclear Green™ LCS1 into 0.5 mL of cell solution
3. Incubate at 37°C, 5% CO_2 incubator for 30 - 60 minutes
4. Analyze cells with a flow cytometer using 530/30 nm filter (FITC channel)

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

KEY PARAMETERS

Flow cytometer

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

CELL PREPARATION

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 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™ LCS1 (Component A) into the treated cells.

4. Incubate the cells 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 intact, and wash the cells once with serum-containing media prior to incubation with Nuclear Green™ LCS1. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment. It is not necessary to fix the cells before DNA staining since the Nuclear Green™ LCS1 is cell-permeable.

5. 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.

6. Monitor the fluorescence intensity using a flow cytometer using 530/30 nm filter (FITC channel). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

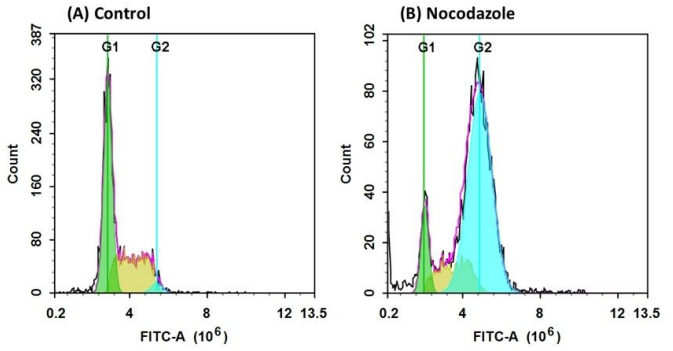

Figure 1. DNA profile in growing and nocodazole treated Jurkat cells. Jurkat cells were treated without (A) or with 100 ng/ml Nocodazole (B) in a 37 °C, 5% CO2 incubator for 24 hours, and then dye loaded with Nuclear Green™ LCS1 for 30 minutes. The fluorescence intensity of Nuclear Green™ LCS1 was measured with ACEA NovoCyte flow cytometer with the channel of FITC. In growing Jurkat cells (A), nuclear stained with Nuclear Green™ LCS1 shows G1, S, and G2 phases. In Nocodazole treated G2 arrested cells (B), frequency of G2 cells increased dramatically and G1, S phase frequency decreased significantly.

DISCLAIMER

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