Cell Meter™ Fluorimetric Fixed Cell Cycle Assay Kit *Red Fluorescence Optimized for Flow Cytometry*

Catalog number: 22842
Unit size: 100 Tests

Component	Storage	Amount
Component A: 100X Nuclear Red™ CCS1	Freeze (<-15 °C), Minimize light exposure	1 vial (250 µL)
Component B: 100X RNase A	Freeze (<-15 °C), Minimize light exposure	1 vial (250 µL)
Component C: 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 Nuclear Red™ CCS1, a cell cycle stain in fixed cells. The dye passes through a permeabilized membrane and intercalates into cellular DNA. The signal intensity of Nuclear Red™ CCS1 is directly proportional to DNA content after RNA is degraded by RNase provided in the kit. 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 monitored with a flow cytometer (FL2 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. Fix cells with 70% Ethanol
3. Add 5 µL of 100X Nuclear Red™ CCS1 and 5 µL of RNase A into 0.5 mL of cells solution
4. Incubate at room temperature for 30 - 60 minutes
5. Analyze cells using a flow cytometer with FL2 channel

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	610/20 nm filter
Instrument specification(s):	PE-Texas Red 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. Treat cells with test compounds for a desired period of time to induce apoptosis or other cell cycle functions.

2. For each sample, prepare cells in 0.5 mL PBS at a density of 5×10^5 to 1×10^6 cells/mL.

   For Adherent Cells: The cells are trypsinized, suspended in 10% FBS medium, centrifuged (1000 rpm, 5 min), and the pellets are resuspended in PBS.

   For Suspension Cells: The cells are centrifuged (1000 rpm, 5 min), and the pellets suspended in PBS (1 mL).

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

Fix the cells with 70% Ethanol:

1. Pipet 0.5 mL cell suspension into 1.2 mL absolute Ethanol (final concentration approx. 70%).

2. Incubate cells on ice for at least 2 hours (or overnight at -20°C). Cells can be stored at -20°C for up to 2 years before staining.

   Note Ethanol is commonly used for fixation after cell surface antigens were stained with monoclonal antibodies, while methanol is commonly used for fixation after intracellular antigens were stained with monoclonal antibodies. In this procedure whole cells are fixed and analyzed. Because the entire cell mass is still present, the use of RNase is typically included to eliminate any double-stranded RNA. Despite the fact that whole cells are being analyzed, attempts to detect some intracellular antigens in conjunction with DNA may fail because the proteins leak out of the permeabilized cell (e.g. green fluorescent protein). In these cases a brief pre-fixation (10 minutes at 4 - 6°C) with 1% paraformaldehyde in PBS before the alcohol fixation will help retain the proteins in the cell.

Stain the cells with Nuclear Red™ CCS1:

1. Pellet the cells at 1000 rpm for 5 minutes and wash cells at least once with cold PBS.

2. Suspend the pellet in 0.5 mL of Assay buffer (Component C).

3. Add 5 µL of 100X Nuclear Red™ CCS1 (Component A) and 5 µL of 100X RNase A (Component B).

4. Incubate the cells at room temperature for 30 to 60 minutes.

   Note 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 5 minutes, and re-suspend cells in 0.5 mL of assay buffer (Component B) or buffer of your choice.

6. Monitor the fluorescence intensity with a flow cytometer using FL2 channel (Ex/Em = 490/620 nm). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

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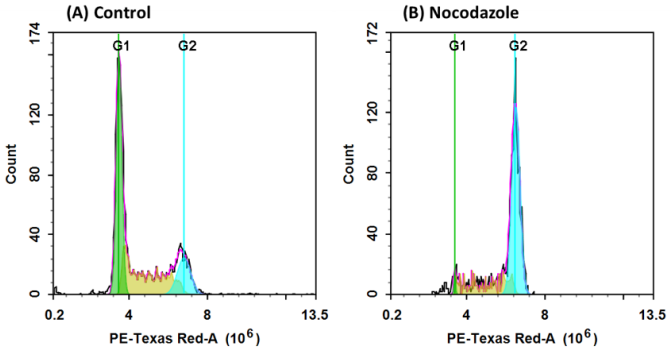

Figure 1.

DNA profile in growing and nocodazole treated Jurkat cells. Jurkat cells were treated without (A) or with 100 ng/ml Nocodazole (B) in 37 °C, 5% CO2 incubator for 24 hours before fixed with 70% ethanol, dye loaded with Nuclear Red™ CCS1 and treated with RNase A were loaded for 30 minutes. The fluorescence intensity of Nuclear Red™ CCS1 was measured with ACEA NovoCyte flow cytometer with the channel of PE-Texas Red. In growing Jurkat cells (A), nuclear stained with Nuclear Red™ CCS1 shows G1, S, and G2 phases (A). In nocodazole treated G2 arrested cells (B), frequency of G2 cells increased dramatically and G1, S phase frequency decreased significantly.