Cell Meter™ Fluorimetric Cell Cycle Assay Kit

Red Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22842 (100 assays)	Keep in freezer and avoid exposure to light	Flow Cytometer

Introduction

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. 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 detecting variations in growth patterns, monitoring apoptosis, and 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 at Ex/Em = 490/620 nm (FL2 channel).

Kit Components

Components	Amount
Component A: 100X Nuclear Red™ CCS1	1 vial (250 µL)
Component B: 100X RNase A	1 vial (250 µL)
Component C: 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 → Fix cells with 70% Ethanol → Add 5 µL of 100 X Nuclear Red™ CCS1 and 5 µL of RNase A into 0.5 mL of cell solution → Incubate at room temperature for 30-60 minutes → Analyze cells with a flow cytometer

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

1. Prepare cells:

1.1. Treat cells with test compounds for a desired period of time to induce apoptosis or other cell cycle functions.

1.2. For each sample, prepare cells in 0.5 mL PBS at a density of 5×10^5 to 1×10^6 cells/mL.
Note 1: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

Note 2: For Adherent Cells: The cells are trypsinized, suspended in 10% FBS medium, centrifuged (1000 rpm, 5 min), and the pellets are resuspended in PBS.

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

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

2. Fix the cells with 70% Ethanol:

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

Note 2: 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.

3. Stain the cells with Nuclear Red™ CCS1:

3.1. Pellet the cells at 1000 rpm for 5 minutes (from Step 2), and wash cells at least once with cold PBS.

3.2. Suspend the pellet in 0.5 mL of Assay buffer (Component C), and add 5 µL of 100X Nuclear Red™ CCS1 (Component A) and 5 µL of 100X RNase A (Component B). 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.

3.3. *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.

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

Data Analysis

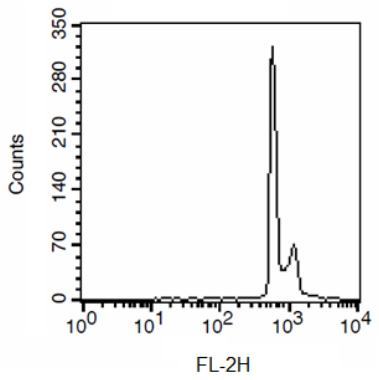

Figure 1. DNA profile in growing Jurkat cells. Jurkat cells were dye-loaded with Nuclear Red™ CCS1 and RNase A for 30 minutes. The fluorescence intensity of Nuclear Red™ CCS1 was measured with the FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using the FL2 channel.

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.