Cell Meter™ Fluorimetric Cell Cycle Assay Kit

Red Fluorescence Optimized for Flow Cytometer

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22860 (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 cells to grow and double the mass of their proteins and organelles. They are also used by cells to monitor internal and external conditions before proceeding with the next phase of cell cycle. The cell's passage through cell cycles 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 Orange™-2 in live 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 Red™ CCS2 can be monitored with a flow cytometer at Ex/Em = 488 nm/615 nm (PE-Texas Red channel).

Kit Components

Components	Amount
Component A: 500X Nuclear Red™ CCS2	1 vial (100 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 1 µL of 500X Nuclear Orange™ into 0.5 mL of cell solution → Incubate at 37 °C, 5% CO_2 for 10-30 minutes → Analyze using a flow cytometer with PE-Texas Red channel (Ex/Em = 488 nm/615 nm)

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.
2. Treat cells with test compounds for a desired period of time to induce apoptosis, cell cycle arrest or other cell cycle functions.
3. Add 1 µL of 500X Nuclear Red™ CCS2 (Component A) in cells containing growth medium, and incubate the cells in a 37 °C, 5% CO_2 incubator for 10 to 30 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 Red™ CCS2.
   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 cells before staining since Nuclear Red™ CCS2 is cell- permeable.
4. *Optional:* Centrifuge cells at 1000 rpm for 4 minutes and re-suspend cells in 0.5 mL of assay buffer (Component B) or a buffer of your choice.
5. Monitor the fluorescence intensity by flow cytometry using the 488 nm laser (Ex/Em = 488/615 nm). Gate the cells of interest, excluding debris.

Data Analysis

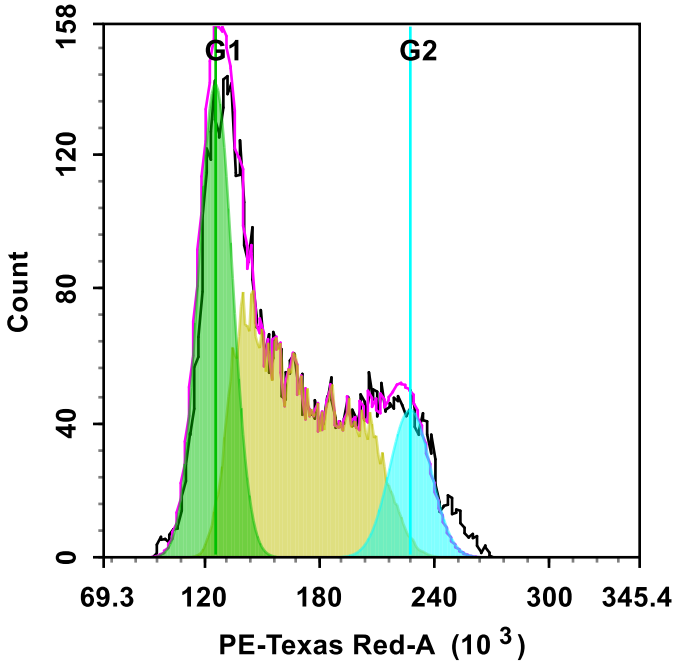

Figure 1. DNA profile in growing Jurkat cells. Jurkat cells were stained with Nuclear Red™ CCS2 for 30 minutes. The fluorescence intensity of Nuclear Red™ CCS2 was measured using ACEA NovoCyte flow cytometer with the channel of PE-Texas Red, the image was generated using Cell Cycle Analysis module of NovoExpress software. In growing Jurkat cells, G0/G1 and G2/M phase histogram peaks are separated by a S-phase distribution.