

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

Catalog number: 22860
Unit size: 100 tests

Component	Storage	Amount
Component A: 500X Nuclear Red™ CCS2	Freeze (<-15 °C), Minimize light exposure	1 Vial (100 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 Bottle (50 mL)

OVERVIEW

Cell cycles have 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 assay kit is designed to monitor cell cycle progression and proliferation by using our proprietary Nuclear Red™ CCS2 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 (PE-Texas Red 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 1 µL of 500X Nuclear Red™ CCS2 into 0.5 mL of cell solution
3. Incubate at 37 °C, 5% CO₂ for 10 - 30 minutes
4. Analyze cells using flow cytometer with PE-Texas Red channel (Ex/Em = 488 nm/615 nm)

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. 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.

4. Incubate the cells in a 37 °C, 5% CO₂ incubator for 10 to 30 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 Red™ CCS2. 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 cells before staining since Nuclear Red™ CCS2 is cell-permeable.

5. **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.
6. Monitor the fluorescence intensity using a flow cytometer with PE-Texas Red channel (Ex/Em = 488/615 nm). Gate the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

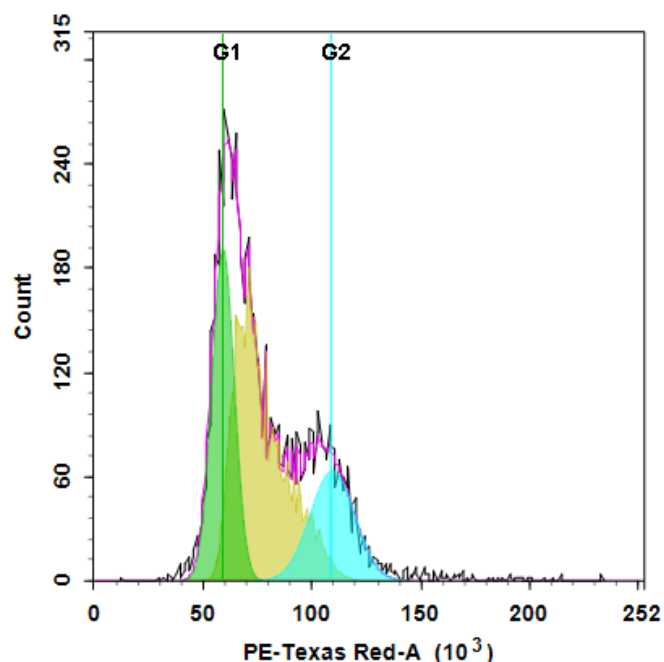


Figure 1.

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.

DISCLAIMER

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