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Product Information

Cell Cycle Analysis Kit

Catalog Number **MAK344**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The cell cycle is a ubiquitous, complex sequence of events leading to growth and proliferation of cells. The cell cycle progression is tightly regulated due to its involvement in development, DNA damage and repair, etc. Anomalies in cell cycle progression can lead to tissue hyperplasia and diseases such as cancer. The cell cycle can be subdivided into interphase (G_0/G_1, S and G_2) and mitotic (M) phase (prophase, metaphase, anaphase, and telophase).

The Cell Cycle Analysis Kit provides a quick and easy method to detect the number of cells in a cell population, which are at a specific stage of the cell cycle. The kit utilizes a nuclear dye, the binding of which to nucleic acids in the cell results in fluorescence signal, which is proportional to cellular DNA content. The percentages of cells in different phases of the cell cycle (G_0/G_1, S, and G_2/M) can be quantified by flow cytometry.

The method is non-radioactive, rapid, and accurate and can be used for high throughput cell cycle analysis with contemporary flow cytometer instruments.

Components

The kit is sufficient for 100 assays.

10× Cell Cycle Assay Buffer Catalog Number MAK344A	50 mL
Enzyme A Solution Catalog Number MAK344B	2 × 250 µL
Nuclear Dye Catalog Number MAK344C	2 × 1 mL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories
- 6-well tissue culture plate
- Cell Culture Media
- Fetal Bovine Serum (Catalog Number F2442)
- Ethyl Alcohol, Pure (Catalog Number 459844)
- Flow Cytometer with excitation filter at 488 nm

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light upon receiving. Warm all reagents to room temperature before use.

For long-term storage of Nuclear Dye, aliquot and store at –20 °C to avoid repeated freeze/thaw.

Preparation Instructions

Reagent Preparation

70% Ethanol – Dilute 7 parts Ethyl Alcohol with 3 parts purified water. Pre-chill on ice prior to use.

1× Cell Cycle Assay Buffer – Dilute10× Cell Cycle Assay Buffer 10-fold with ultrapure water to prepare 1× working solution. Pre-chill on ice before use.

Staining Solution – For every 20 samples (based on 6 well plate sample size) being analyzed, add 100 µL of Enzyme A Solution and 400 µL of Nuclear Dye into 10 mL of 1× Cell Cycle Assay Buffer, mix well, and protect from light. Stable for one week at 2–8 °C.

Sample Preparation

1. Grow cells of interest (2–5 × 10^5 cells/well) in desired medium and culture conditions preferably in 6 well plates for 24 hours prior to the experiment.
2. Synchronize cells with culture medium containing 0.1% FBS for 24 hours.
3. Treat cells with test compounds in culture medium containing 10% FBS for 4–24 hours. As controls, incubate cells of interest in culture medium with 10% FBS without any test compound.
4. Harvest cells and centrifuge at 400 × *g* for 5 minutes.
5. Remove the supernatant and wash cells in 2 mL of ice cold 1× Cell Cycle Assay Buffer.
6. Centrifuge cells at 400 × *g* for 5 minutes.
7. Remove the supernatant and save the cell pellet.

Notes: Cell density depends on the cell type, and it may be necessary to adjust the cell numbers for optimal cell density. For longer incubation times, change culture medium containing 10 % FBS with test compounds every 24 hours.

Procedure

Nucleic Acid Labeling

1. Fix the cells by adding 2 mL ice cold 70% ethanol (add drop by drop while vortexing) to the cell pellet. After fixing, cells can be stored at –20 °C for several weeks in 70% ethanol.
2. Place on ice for a minimum of 30 minutes.
3. Centrifuge cells at 400 × *g* for 5 minutes.
4. Carefully remove the supernatant.
5. Wash cells in 2 mL of 1× Cell Cycle Assay Buffer.
6. Centrifuge cells at 400 × *g* for 5 minutes.
7. Carefully remove the supernatant.
8. Resuspend cells completely with 500 µL of Staining Solution, protect from light exposure.
9. Incubate at room temperature for 30 minutes.

Note: After fixing in ethanol, cells are harder to pellet. It is recommended to remove supernatant carefully to avoid cell loss.

Data Analysis – During flow cytometry data analysis, select the main cell population in the FSC vs SSC plot. Within the main cell population, exclude the cell debris and cell aggregates by gating on single cells in the FL2-A vs FL2-W plot. Cell aggregates should have higher value of FL2-W than the main single cell population. Cell cycle analysis is performed with FL2-A histogram of single cells. Cell cycle status can be quantified by programs within the flow cytometer software or gating on the FL2-A histogram.

Figures 1A and 1B.

3T3 cells were seeded at 2 × 10^5 cells/well into a 6 well tissue culture plate with 10% FBS culture medium on day 1. On day 2, medium was removed and cells were synchronized with 0.1% FBS culture medium for 24 hours. On day 3, 3T3 cells were incubated in culture medium with either 0.1% FBS, 10% FBS, or 10% FBS containing 20 µM of SKPin C_1 for an additional 24 hours. On day 4, cells were harvested, fixed, and stained with the Cell Cycle Analysis Kit according to the protocol. Fluorescence intensity was detected and recorded on a BD Flow Cytometer in FL-2 channel.

Figure 1A.

Cell cycle analysis of 3T3 cells in 10% FBS culture medium.

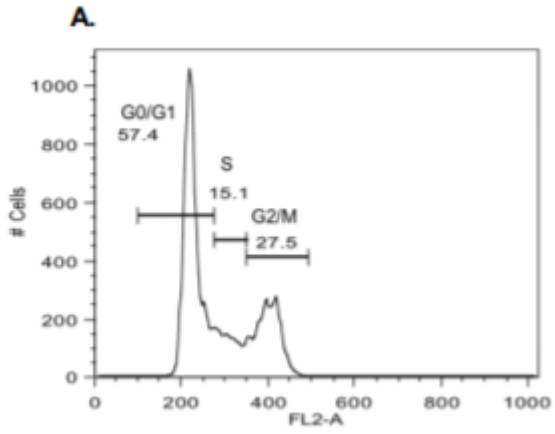
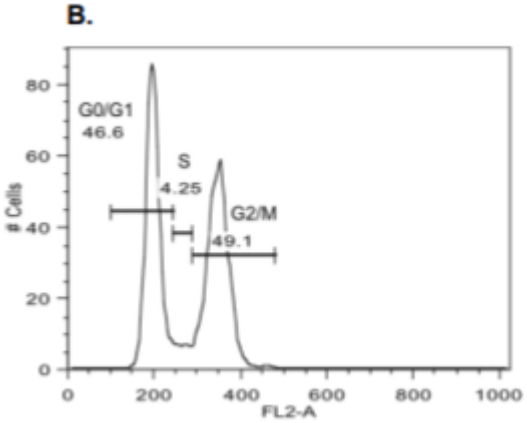


Figure 1B.

Cell cycle analysis of 3T3 cells in 10% FBS culture medium containing 20 µM of SKPin C1.



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