ab287852 – Cell Cycle Analysis Kit

For the quanitative monitoring of cell cycle progression in a cell population.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287852

Storage and Stability

Store the kit at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
10X Cell Cycle Assay Buffer	50 mL	-20°C
Enzyme A Solution	2 x 250 µL	-20°C
Nuclear Dye I	2 x 1 mL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 6-well tissue culture plate
- Cell Culture Media and Fetal Bovine Serum
- 70% Ethanol (Pre-chilled on ice)
- Flow Cytometer with excitation filter at 488 nm wavelength and emission filter FL2 (585/40 nm).

Reagent Preparation

- Warm all reagents to room temperature (RT) before use.
- Read the entire protocol before performing the assay.

10X Cell Cycle Assay Buffer: Dilute 10X Cell Cycle Assay Buffer with ddH2O to prepare 1X working solution. Pre-chill 1X Cell Cycle Assay Buffer on ice before use.

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

Staining Solution: Before performing the analysis, prepare Staining Solution - for every 20 samples (based on 6-well plate sample size): add 100 µl of Enzyme A Solution and 400 µl of Nuclear Dye I into 10 ml of 1X Cell Cycle Assay Buffer, mix well and protect from light. t

Analysis Protocol

Sample Preparation:

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

Δ Note: *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 hr.*

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Nucleic Acid Labelling:

1. Fix the cells by adding 2 ml ice cold 70% ethanol (add drop by drop while vortexing) to the cell pellet, put on ice for at least 30 min.
2. Centrifuge cells at 400 x g for 5 min. and carefully remove the supernatant.
3. Wash cells in 2 ml of 1X Cell Cycle Assay Buffer, centrifuge cells at 400 x g for 5 min. and carefully remove the supernatant.
4. Resuspend cells completely with 500 µl of Staining Solution, protect from light exposure.
5. Incubate at RT for 30 min.

Δ Note: *After fixing in ethanol, cells are harder to pellet. We recommend removing supernatant carefully to avoid cell loss.*

Δ Note: *After fixing, cells can be stored at -20°C for several weeks in 70% ethanol.*

Data Analysis:

1. During flow cytometry data analysis, select the main cell population in the FSC vs SSC plot.
2. 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.
3. Cell aggregates should have higher value of FL2-W than the main single cell population.
4. Cell cycle analysis is performed with FL2-A histogram of single cells.
5. Cell cycle status can be quantified by programs within the flow cytometer software or gating on the FL2-A histogram.

Technical Support

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