THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

CELL CYCLE GUIDELINES

PROCEDURE

1.0 Preparation

1.1 Prepare cells into a single cell suspension. Ensure that the final wash is done with PBS. Cells should be concentrated to approximately 1 million cells in 200 µl.

1.2 Cells should be fixed if possible. However, if a live assay will be performed prior to cell cycle analysis, cell cycle can be determined afterwards on live cells.

1.3 Always include a tube of normally cycling untreated cells as a control.

2.0 Cell Cycle- Fixed

2.1 Ethanol should be used to fix and permeabilize cells whenever possible. If you will also be detecting fluorescent proteins or surface proteins, cells should be fixed with 1% formaldehyde.

2.2 **Fixing with Ethanol**: Add ice cold 70% ethanol drop wise to the cell pellet. Vortex cells while adding ethanol to prevent aggregation. Incubate cells for 30 minutes to overnight at 4°C.

Note: Ethanol should be diluted in diH_2O.

2.3 **Fixing with Formaldehyde**: Add 1 ml of 1% formaldehyde. Incubate for 30 minutes to overnight at 4°C.

2.4 Wash with 1X PBS, centrifuge at 400-450 g for 5 minutes at 4°C, and decant or aspirate supernatant. Repeat wash.

2.5 Add 200 µl of PI+RNase solution to each tube of cells.

Note: PI+RNase solution is 2 ml of 1 mg/ml PI, 1 ml of 10 µg/ml RNase, and 47 ml of 1X PBS.

3.0 Cell Cycle- Live

3.1 Following any live assay data acquisition, add 200 µl of nim-DAPI (NPE Systems Cat #731085) to each tube of cells.

4.0 Analysis

4.1 Analyze data using a software that has a cell cycle modeling program such as FCS Express or FlowJo (version 10 or later).

RELATED PROCEDURES

This handout is related to ACSF SOP EX003. It is a handout only.