

7AAD Cell Cycle Profile of Non-Fixed Cells

Background

This method allows the analysis of cell cycle profile of live cells stained for specific cell surface receptors.

This protocol does not work with GFP labelled cells!

Materials

1. **Fluorochromes conjugated antibodies.** Used to stain markers of interest
2. **Cells.** These will need to be counted and in suspension
3. **7AAD/Saponin solution:** 0.03% Saponin, 25µg/ml 7AAD, 1% BSA. This solution can be made in PBS (e.g. for Jurkat and most cell lines) or 10mM HEPES (e.g. for thymocytes and cells too sensitive to changes in pH).

Additional Considerations

1. **Single colour control:** If you're planning to label cells with 2 or more antibodies simultaneously, you need a single colour control for each fluorochrome. If you have a limited number of cells there are alternatives that use beads, just ask and we can assist you.
2. **Negative Sample:** An amount of unstained cells/sample used to initially adjust settings on the machine

Equipment

1. **Centrifuge.**
2. **Pipettes.** You will need two: one in the range of 10-100µl, and another ranging from 100-1000µl.
3. **12x75 mm polystyrene/polypropylene tubes.** Depending on which machine you wish to use (LSR II prefers polystyrene while the CyAn prefers polypropylene).
4. **Ice bucket with cover.** Generally, cells are more stable and tolerate insult better when they're cold. The cover keeps light out, which could bleach the fluorochromes.
5. **Flow cytometer.** We have a variety of machines at your disposal including a BD LSR II, BD FACSAria and a Beckman Coulter CyAn. Each machine has different capabilities, strengths and weaknesses so be sure to check with us which machine is suited for your needs.

Procedure

1. Harvest cells and stain desired cell surface markers. You can use all fluorescence channels except FL4 (Cyan)/PE-Cy5 (LSR II), which should be kept for detection of 7AAD fluorescence. For cell surface staining you can follow any standard protocol, e.g. stain cells with appropriate labelled antibodies for 20 min on ice in PBS/1% FCS buffer.
2. Wash once with PBS and resuspend cells in 7AAD/Saponin solution. Guideline: 400µl per up to 5 million cells.

3. Incubate at 37°C for 30 to 60 minutes. The incubation time can vary according to cell type.
4. Transfer cells onto ice until analysis (no need to wash)
5. If you need to dilute samples while acquiring data, remember to dilute with 7AAD/Saponin solution to keep 7AAD and Saponin concentrations the same.

Flow analysis:

Keep the cells at on ice covered until your scheduled time on the flow cytometer.

When analysing samples, be sure to collect 7AAD in linear scale. Use a dot plot showing 7AAD parameter Area vs Height (LSRII)/Peak (CyAn) or Width (LSRII) to gate out doublets and clumps and analyse at a low flow rate under 400 events/second.