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.

Materials

1. **Fluorochromes conjugated antibodies.** Used to stain makers of interest
2. **Cells.** These will needed to be counted and in suspension
3. **7AAD/Saponin solution**: 0.03% Saponin, 25 g/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.**

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3. Incubate at 37^{o}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.

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