BrdU Staining Using DNAse I

Background

This method is for the investigation of cell proliferation via detection of BrdU incorporation. BrdU (Bromodeoxyuridine) is a thymidine analogue that is readily incorporated into newly synthesized DNA by cells progressing through DNA synthesis (S) phase and cell cycle. Using an anti-BrdU antibody, S phase cells can then be detected. The standard BrdU protocol uses acid treatment to unwind the DNA to help the anti-BrdU antibody access DNA incorporated BrdU. The harsh acid treatment doesn't allow using this method in combination with immunophenotyping or with GFP labelled cells. Replacing the acid with DNase I to unwind the DNA allows proliferation study in cells stained for specific cell surface markers or in GFP positive cells

Materials

- **BrdU** Sigma B5002): (Dissolve 30.7mg BrdU from Sigma in 90ml of dH2O, add 10ml 10X PBS and filter sterilize with 0.22μm filter. This makes 1mM BrdU solution stock. Aliquot and freeze down at -20°C until needed) stock. Aliquot and freeze down at -20°C until needed)
- **Anti-BrdU** (BD 347580)
- **Seconday flurochrome conjugated antibody** i.e. goat anti-mouse AlexaFluor 647
- **1-4% Paraformaldehyde**
- **PBS**
- **0.5% Triton x-100**
- **RQ1 RNase-Free DNase** (Promega M6101)
- **PBS-T** (PBS, 0.2% tween20, and 1% BSA)
- **RNaseA** 100μg/ml
- **PI** 50μg/ml

Additional Considerations

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

Equipment

1. **Centrifuge.**
2. **Pipettes.** You will need three: one in the range of 2-10μl, one in the range of 10-100μl, and another one 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 you disposable including a BD LSR II, BD FACSArray and a Beckman Coulter CyAn. Each machine has different capabilities, strengths and weakness so be sure to check with us which machine is suited for your needs.

Procedure

1. Treat cells with 10μM BrdU for an appropriate time (30 minutes-48 hours).
2. Stain cells for cell surface antigen (optional if you are only using GFP cells).
3. Fix cells with 1-4% PFA on ice for 10 min. Cells can be kept overnight at 4^0C.
4. Pellet cells and decant the supernatants being careful not to disturb the pellet.
5. Add 1ml PBS and wash twice as above.
6. Permeabilise cells by adding 500μl of 0.5% Triton X-100 in PBS, incubate for 15 min at room temperature.
7. Pellet and wash twice with PBS.
8. Resuspend cells in 500μl of 1 X DNase I buffer provided with the enzyme. Add 25 to 50μl DNase I solution.
9. Incubate at 37^o for 45 min.
10. Pellet and wash 3 times with PBS-T.
11. Add 2μl of anti-BrdU and incubate for 20 min at room temperature.
12. Pellet and wash once with PBS-T.
13. Add 50μl of an appropriate fluorochrome conjugated anti-mouse at 1 /10 dilution in PBS-T, incubate in the dark for 20 min at room temperature.
14. Wash once with PBS.
15. Resuspend cells in 50μl RNaseA (100μg/ml) and 200μl PI (50μg/ml) and incubate for 5-15 minutes.
16. Keep the cells covered at RT until your scheduled time on the flow cytometer. Save at least 20,000 single cells.

Flow analysis:

- When analysing samples, be sure to collect PI in linear scale. Use a dot plot showing 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.
- A dot plot showing PI on X-axis and FITC-anti-BrdU on Y-axis should show a horseshoe cell distribution and allows distinction of cells in G1, S, and G2M phases.

Flow Cytometry Core Facility, Camelia Botnar Laboratories, Room P3.016
UCL Institute of Child Health. 30 Guilford Street, London WC1N 1EH
Tel 020 7405 9200 ext 0198