BrdU Staining Using Acid Treatment

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 in cell cycle. Using an anti-BrdU antibody, S phase cells can then be detected. This standard BrdU protocol uses acid treatment to unwind the DNA to help the anti-BrdU anti-body access DNA incorporated BrdU and therefore cannot be used with immunophenotyping or GFP cells. Refer to BrdU protocol using DNAseI for these applications.

Materials

1. **BrdU** (Sigma B5002): (Dissolve 30.7mg BrdU from Sigma in 90ml of dH_2O, add 10ml 10x PBS and filter to sterilize with 0.22μm filter. This makes 1mM BrdU solution stock. Aliquot and freeze down at -20°C until needed)
2. **Anti-BrdU** (BD 347580)
3. **FITC-conjugated goat anti-mouse F(ab')2 fragments** (Sigma F2653)
4. **70% ethanol** (in DI water)
5. **2M Hydrochloric Acid** (AppliChem A2427) (add 1 part 37% concentrated HCl to 4 parts distilled water).
6. **PBS**
7. **PBS-T** (PBS, 0.2% tween20, and 1% BSA)
8. **RNaseA** 100μg/ml
9. **PI** 50μg/ml

Additional Considerations

1. **Negative control**: A million of unlabeled cells stained with anti-BrdU the same way as the BrdU labelled cells.
2. **Positive control:** exponentially growing cells stained for BrdU incorporation

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.

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Procedure

1. Treat cells with 10μM BrdU for an appropriate time (30 minutes to 48 hours).
2. Harvest cells and wash off remaining BrdU.
3. Fix cells in cold (4°C) 70% ethanol. Mix while fixing to prevent excess clumping and to ensure adequate fixation of all cells. Leave for at least 30 minutes on ice. Samples may be left at this stage for up to a week.
4. Pellet cells at higher speed compared to live cells, decant supernatants being careful not to lose the pellet. Loosen pellet before adding PBS.
5. Add 1ml PBS and wash twice as above.
6. Re-suspend cells in 1ml 2M hydrochloric acid. Leave at room temperature for 30 minutes, mixing at intervals.
7. Pellet and wash twice with PBS.
8. Wash once with PBS-T.
9. Add 2μl anti-BrdU antibody directly to the cell pellet and incubate for 20 minutes at room temperature in the dark. The longer the incubation time, the stronger the signal.
10. Wash once with PBS-T
11. Stain with 50μl FITC-conjugated rabbit anti-mouse F(ab')2 fragments at 1 in 10 dilution in PBS-T for 20 minutes at room temperature in the dark.
12. Wash once with PBS.
13. Resuspend cells in 50μl RNaseA and 300μl PI and incubate for 5-15 minutes.
14. Keep the cells at RT covered, 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.

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