

JHM
9/14/04

BrdU Staining Protocol

Supplies:

Ethanol 100% USP (highest quality)
FACS Staining Buffer (1XPBS w/ 3% calf serum, 0.05% azide--filtered)—Dilute staining antibodies in Buffer
DNase (Sigma D-5025, Bovine Pancreas)
RNase (Boehringer, 25 mg bovine pancrease)
Anti-BrdU-FITC (Becton Dickinson or Phoenix Flow)
0.15 M NaCl, 1.5 M NaCl
10% Paraformaldehyde (kept as stock in -80C)
Tween
1M MgCl₂
FACS Tubes

Protocol:

Cells in 96 well FACS plate

1. Block with 24G-2
 2. Surface stain cells as usual
 - Omit** fourth channel labeled antibodies on **all** stains; **EtOH destroys APC**
 3. Prepare tubes from which to transfer EtOH drop wise (1.2 ml EtOH on ICE)
 4. Resuspend cells from 96 well place with 100 μ l 0.15M NaCl (**cold**)
 5. Transfer to FACS tubes ON ICE. Add 400 μ l 0.15M NaCl to each tube
 6. Vortex at 1/3 speed and add EtOH with pasteur pipette at 1 drop per second.
 - This is a critical step...** do not add EtOH too quickly
 7. Incubate on ice for 30 minutes
 8. Spin 10 minutes @ **2000 RPM, 4° C**
 9. Dump and shake liquid into waste
 10. Using repeat pipetter, squirt 1 ml FACS staining buffer into each tube
 11. Spin 10 minutes and dump as before (step 8)
 12. Add 1 ml 1% paraformaldehyde + 0.05% Tween 10
 - For 20 ml:
 - 2.0 ml 10% paraformaldehyde
 - 10 μ l Tween-20
 13. Incubate at **room temperature** for 30 minutes
 14. Incubate **on ice** for 30 minutes
 15. Spin and dump as before (step 8)
- Add 1 ml DNase (0.15M NaCl + 4.2mM MgCl + 100 Kunitz units/ml DNase)
- For 50 ml:
 - 46.5 mL dH₂O
 - 200 μ l MgCl₂ (1M stock)
 - 1500 μ l NaCl (5M stock)
 - 100 Kunitz units Dnase (volume depends on activity of batch)
 - Incubate for 30 minutes @ 25°
16. Spin 10 min. and dump as before (step 8)
 17. Transfer cells from FACS tubes to 96-well plate. Wash once with staining media
 18. Block with 10% rat serum. Incubate 15 minute on ice. Spin and dump as before (step 8: it is critical to spin at high speed once the cells have been fixed with EtoH/ PFA since they become less dense).
 19. Add **anti-BrdU-FITC or biotin (1:20 dilution for Phoenix flow)**.
 20. Pipette up and down to resuspend pellet. Incubate for 30 minutes on ice (or overnight at 4C).
 21. Wash and dump as before. Transfer cells into FACS tubes.