

Multiparameter Cell Cycle Analysis002

11/23/10

Notes and Details: This experiment will be a repeat of 001 to determine if our new Gallios target channels are appropriate for the A488 to remain on scale. Also, the experiment will be permed in parallel with methanol and saponin to evaluate permeability issues. A tube will also be stained with H3 A647 for possible staining panel changes. This SOP was modified from the Jacobberger SOP for the flow cytometry book

Initials: MEP/SLB

Cell Culture Preparation

1. Molt-4 cells had previously been in culture and split 1 day earlier.
2. Count cells using AcT10; 20 μ L of sample into prediluted counting tube
3. Place 2×10^6 cells per tube for flow cytometry staining
4. Centrifuge at 400g for 7min at RT
5. Decant supernatant
6. Process samples by Methanol or Saponin procedures

FIXATION & Methanol PERMEABILIZATION

1. Add 1.5mL of 1xPBS and 0.5mL 4% formaldehyde to each sample to make a 1% solution
2. Incubate 20 mins. @ R.T.
3. Centrifuge at 400g for 7min at RT
4. Wash with 1mL cold PBS
5. Centrifuge at 400g for 7min at RT
6. Resuspend in 50 μ L cold PBS
7. Add 450 μ L MeOH (stored at -20 $^{\circ}$ C)
8. Allow 1h on ice for permeabilization
9. Centrifuge at 400g for 7min at RT
10. Wash twice with 1mL cold PBS
11. Centrifuge at 400g for 7min at RT
12. Wash with 0.5 mL PBS-2% BSA (cold) to begin blocking process
13. Add antibodies as per STAINING section
14. Incubate at 37C for 30-90min
15. Cool to 4C by placing in refrigerator for ~5min
16. Wash twice with 500uL of PBS/2%BSA cold
17. Resuspend in 1ug/mL DAPI + PBS (500uL)
18. Acquire

FIXATION & Saponin PERMEABILIZATION

1. Fix with 100 μ L of PBS-A + 100 μ L of 4% formaldehyde in hypertonic PBS-A
2. Incubate for 20 minutes @RT
3. Wash 1X PBS/0.5%BSA/Azide

4. Add 150 μ L/well PBS/0.5%BSA/Saponin, mix well
5. Incubate 10 minutes @ RT
6. Centrifuge at 400g for 7min at RT
7. Add antibodies as per STAINING section
8. Incubate 30 minutes @RT
9. Wash 1X PBS/0.5%BSA/Saponin
10. Wash 1X PBS/0.5%BSA/Azide
11. Resuspend in 1ug/mL DAPI + PBS (500uL)
12. Acquire

STAINING

1. Resuspend the pellets in the following antibody cocktails:
 - a. Cyclin B1 A647: [stock] 100ug/2mL [final] 0.06ug/50uL (1.2uL in 50uL)
 - b. Cyclin A2 PE: [stock] 0.125ug/uL [final] 0.125ug/50uL (1uL in 50uL)
 - c. Phospho-S10-histone H3 A488: [stock] 37.5ug/mL [final] 0.125ug/50uL (0.667uL in 100uL **two tests**)
 - d. DAPI (not to be added until acquisition): [stock] 200ug/mL [final] 1ug/mL in PBS (2.5mL PBS + 12.5uL DAPI)

Adapted from procedures, complements of Donnenberg Lab, University of Pittsburgh