BRDU Staining

Materials

- PBS N3. Add 5.0 ml of 1M NaN3 to 495 ml PBS.
- PBS-FCS N3. Add 5.0 ml NaN3, 5.0 ml of 3.5%NaHCO3 soln and 25 ml heat inactivated fetal bovine serum to 465 ml PBS. Store at 4 °C.
- Fixative (1% formaldehyde). Add a 10 ml vial of 20 % EM grade formaldehyde to 190 ml PBS. Store at 4 °C. • BectonDickinson Lysing solution. Dilute to 1X with deionized water.
- Perm solution. Measure 450 µl PBS, warm to room temp, add 50 µl NP40 using a shortened pipette tip (it is viscous), vortex until fully dissolved. Take 250 µl of this and add it to 50 mlof fixative.
- DNase solution. Use Boehringer Mannheim Dnase, add 2.5 ml 10X buffer, 22.5 ml deionized water and125 µl Dnase, store at -80 °C. • NP40-FCS/PBS solution. Add 100 µl NP40 to 20 ml PBS-FCS N3 soln, vortex until dissolved.
- NP40/ PBS solution. Add 100 µl NP40 to 20 ml PBS, vortex until dissolved.
- Antibodies. 1st is the surface staining cocktails, 2nd is the Streptavidin second step ifany, and 3rd is the BRDU FITC.
- Tubes. We use12X75 mm polystyrene conical bottom tubes (from Fisher).

Method day 1

1. Aliquot samples into test tubes, add 1 ml of PBS N3, centrifuge at 1800 rpm for 10 minutes, aspirate all but 20 µl of your supernatent, resuspend cell pellet.
2. Add the surface cocktails, mix andincubate on ice in the dark for 30 minutes.
3. Add 1 ml PBS N3, centrifuge at 1500 rpm at 4 °C for 7 minutes, aspirate all but 20 µl supernatant, resuspend cells.
4. Add 2nd antibody, eg Streptavidin Per CP where appropriate, mix, incubate for 15 minutes on ice in the dark.
5. Wash by adding 1 ml PBS N3, centrifuge at 1500 rpm at 4 °C for 10 minutes.
6. Add 900 µl of 1X BD FACS Lysing solution, incubate at room temperature in the dark for 15 minutes.
7. Centrifuge at 2300 rpm at 4 °C for 10 minutes, aspirate supernatant resuspend pellet.
8. Add 1 ml of Perm solution and vortex immediately, store in the refrigerator overnight.

Method day 2

1. Centrifuge tubes at 2300 rpm at 4 °C for 10 minutes, aspirate supernatant, resuspend cells.
2. Add 1 ml PBS, centrifuge at 2300 rpm at 4 °C for 15 minutes, aspirate supernatant, resuspend cells.
3. Add 1 ml of Dnase solution and vortex, incubate at 37 °C for no more than 30 minutes.
4. Centrifuge at 2300 rpm at 4 °C for 10 minutes, aspirate supernatant resuspend pellet.
5. Add 1 ml NP40-FCS/PBS, centrifuge at 2300 rpm at 4 °C for 10 minutes, aspirate all but 20 µl of supernatant resuspend pellet.
6. Add 5 µl Becton Dickinson anti-BRDUFITC or control antibody, incubate for 45 minutes at 4 °C in the dark.
7. Add 1 ml NP40-FCS/PBS, centrifuge at 2300 rpm at 4 °C for 10 minutes, aspirate supernatant, resuspend pellet.
8. Add 1 ml NP40/PBS, centrifuge at 2300 rpm at 4 °C for 10 minutes, aspirate all but 20 µl of supernatant resuspend pellet.
9. Add 300 µl of freshly made formaldehyde solution while vortexing.