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BrdU FACS Protocol

1. Inject mice IP with 200 μ l 10 mg/ml BrdU (BD FITC BrdU Flow Kit, cat # 559619; stored at -80°C ; 2 mg BrdU per mouse) 3 hours prior to sacrifice.
2. Sacrifice, harvest, crush, lyse and count per usual.
3. Surface stain, wash with FACS buffer, spin and pour off supernatant.
4. Fix and permeabilize cells by resuspending with 100 μ l BD Cytofix/Cytoperm Buffer (same kit, stored at 4°C) per tube. Incubate on ice for 20 minutes. Wash with 1 ml 1X BD Perm/Wash Buffer (same kit) per tube, spin and pour off supernatant.
5. Resuspend cells with 100 μ l BD Cytoperm Plus Buffer (same kit) per tube. Incubate on ice for 10 minutes. Wash with 1 ml 1X BD Perm/Wash Buffer per tube, spin and pour off supernatant.
6. Re-fix cells by resuspending with 100 μ l BD Cytofix/Cytoperm Buffer (same as in step 4) per tube. Incubate on ice for 5 minutes. Wash with 1 ml 1X BD Perm/Wash Buffer per tube, spin and pour off supernatant.
7. Treat cells with DNase to expose incorporated BrdU by dissolving 300 μ l (1 vial, same kit; stored at -80°C) of DNase in 700 μ l sterile PBS and resuspend cells with 100 μ l per tube. Incubate at 37°C for 1 hour. Wash with 1 ml 1X BD Perm/Wash Buffer per tube, spin and pour off supernatant.
8. Add 5 μ l anti-BrdU FITC (eBioscience cat # 11-6071-42, clone PRB-1; ****NOTE: DO NOT USE THE ANTIBODY THAT COMES WITH THE BD BRDU KIT! IT DOES NOT WORK!****) per tube. Incubate at room temperature for 20 minutes. Wash with 1X BD Perm/Wash or FACS buffer, spin and pour off supernatant.
9. Store overnight at 4°C .
10. FACS.