

## Simultaneous Surface- and DNA Content-Staining for FACS

protocol by Susannah D. Barbee

1. Surface stain with FITC- and PE-conjugated antibodies.
2. Wash 2x 4 mL PBS (Cf 6-10 min 1200 rpm).
3. Resuspend in 875  $\mu$ L PBS/0.1% Na Azide.
4. Add 125  $\mu$ L 2% paraformaldehyde, and **vortex immediately**.
5. On ice 1 hr; Cf and aspirate.
6. Resuspend in 1 mL PBS + 0.05% Tween 20 (**RT**).
7. Incubate 15 min 37°C.
8. Add 1 mL PBS/0.1% Na Azide, Cf. Repeat 2x.
9. Resuspend in 1 mL 25  $\mu$ g/mL 7AAD (Molecular Probes A1410) in PBS/0.1% Na Azide.
10. On ice  $\geq$ 30 min; analyze within 2 hrs.

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