

Intracellular Staining of Bcl-2 for FACS

protocol by Susannah D. Barbee

1. Aliquot $\sim 2 \times 10^6$ cells per well in a 96-well plate. Stain with PE- and CyChrome-conjugated antibodies; wash 2x 200 μ L PFA (Cf 6-10 min 1200 rpm).
2. Resuspend in 200 μ L Cytotfix/Cytoperm (Pharmingen 2075KK).
3. On ice 30-40 min.
4. Wash 1x 250 μ L PFA.
5. Resuspend in 100 μ L:
80 μ L Perm/Wash (Pharmingen 2075KK)
20 μ L Bcl-2 FITC or isotope control (Pharmingen 1502KK)
6. On ice 30-40 min.
7. Add 150 μ L Perm/Wash, Cf. Wash 2x 250 μ L Perm/Wash†.
8. Resuspend in 400 μ L PFA and analyze.

PFA: 1x PBS
1% FCS
0.01% Na Azide

† The Cytotfix/Cytoperm system is a reversible saponin-mediated permeabilization. Washes must be performed with Perm/Wash to keep cells permeable and to wash out excess antibody.

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