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FACS Staining Protocol

Materials

Reagents of known titration (λ per 45λ rxn volume)

Cells, washed, adjusted to 2.5-5 x 10^7/ml or 5-10 x 10^5/well
3911 Falcon flexible plastic plate
Staining media (SM): PBS/3% FCS/0.04% NaN3. keep on ice.
Centrifuge that can spin plates
Ice
Falcon 2052 tubes

Procedure:

1. Make up staining mixes, and second step e.g.

Reagent	λ/rxn	# rxns	total vol
RS3.1-bi	0.3	10	3.3
a-mu-fl1	10	10	
SM	25 total	10	250-10-3.3=237

Should make up one more rxn than needed. Note, remember to include single color and unstained controls. The single color controls should not get PI. Make one unstained tube with PI and one without.

2. Spin at 4° C about 13K xg for 20-30 min to deaggreagate.

3. Wash cells into SM. Adjust cell count to 2.5-5 * 10^7/ml. Can also aliquot approx. number of cells directly to plate, then wash with 2 x 100λ of SM, reconstituting in about 20λ

4. If preparing cells separately, add 25λ of rxn mix to plate first. Then add 20λ of cells to each appropriate well, mixing and changing tip as you go. By using a crossing pattern, can add same stains to various cell preps or various cell preps to same stains.

5. If preparing cells in plate, add stains directly after cells are washed and reconstituted.

6. On ice for 20', covered with foil.

7. Add 100λ/well of SM, using multichannel pipettor. Spin 1500 rpm x 3' at 4° C.

8. Suck off sup using 9" pasteur hooked to gentle suction. Tilt plate at 45° angle to visualize pellet. Enter the tip of pipette into well at a 45° angle along the edge. Do not try to suck dry.

9. Tap plate gently to dislodge pellets. Add 100λ SM. Spin. Suck. Wash 1x more again.

10. If using streptavidin or second step, add 50λ/well, mixing and changing tips as you go. This should have been prepared in #1 above. Otherwise, skip to step 13.

11. On ice for 15', covered.

12. Wash as in steps 7-9.

13. Final resuspension in 100λ of SM. Transfer to labelled (I use the 96wp grid A-H, 1-12 for labels) 2052 tubes which contain 200λ of SM. If using PI, then add at appropriate concentration (e.g. 2ug/ml final- dilute 1:500 of our stock) to the SM before dispensing to

the 2052 tubes. Transfer cells to the 2052 tubes. Keep on ice and in the dark. Analyze on FACS