General Surface Staining Protocol

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Materials	Qty	Order Info
Staining Media		
Fixing Solution		
Antibody		
FACS tubes		Falcon 35-2052 (no caps), 2058 (caps)
4% Paraformaldehyde		
Large ice bucket		
96-well plates		
Aluminum Plate Holders		

Basic staining paradigm: 50 ul stain + 50 ul cells

1. Resuspend 0.5-1 million cells in 50 ul staining media for each stain.
2. Make up the stain: use the recommended titre of each reagent and dilute with staining media to 50 ul.
3. Clearly **label** all wells or FACS tubes.
4. Add the cells to the stain (total vol= 100 ul) and incubate for 15 min on ice.
5. Add 150 ul staining media and centrifuge.
6. Aspirate, add 200 ul staining media and centrifuge.
7. Repeat step 5 (2 1/2 washes = 3 spins total).
8. Aspirate and resuspend in 200 ul fixing solution.
9. Transfer to a FACS tube.
10. Cells may be stored covered at 4C until analysis.

Staining multiple samples

1. For speed and convenience, samples can be stained in a 96 well plate (see diagram below). Place **stains** in alternating wells across a row (marked **X** below)
2. Place **cells** in alternating wells next to the stains (marked **O**, below)

   Note: Stain combinations typically go across a row (1,3,5,...) while samples go down a column (A,B,C,...). Wells receiving the same staining combination may be placed next to each other, but wells containing different staining combinations should be separated by one empty well.
3. Transfer cells into stains with a multi-channel pipettor using alternating tips.
4. Place plate on a metal holder and incubate for 15 min covered on ice.
5. Wash according to the steps outlined above using a multi-channel pipettor.
6. Transfer to FACS tubes arranged in a rack corresponding to the layout of the 96 well plate.

96 well plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	X	O	X	O	X	O	X	O	X	O	X	O
B	X	O	X	O	X	O	X	O	X	O	X	O
C	X	O	X	O	X	O	X	O	X	O	X	O

D	X	O	X	O	X	O	X	O	X	O	X	O
E	X	O	X	O	X	O	X	O	X	O	X	O
F	X	O	X	O	X	O	X	O	X	O	X	O
G	X	O	X	O	X	O	X	O	X	O	X	O
H	X	O	X	O	X	O	X	O	X	O	X	O

FACS tube staining option

1. For fewer samples, staining in a FACS tube may be faster. This allows for fewer spins -- only one large volume wash is needed.
2. Place stain in the bottom of a FACS tube on ice.
3. Add cells directly to the stain, mix well and incubate on ice for 15 min.
4. Add 4 ml of staining media and centrifuge.
5. Aspirate cells and resuspend in 200 ul fixing solution.
6. Store cells covered at 4C until analysis.

Recipes:

Staining media: deficient hRPMI
3% NCS
0.02% Azide (1/500 of 10% stock)
optional: 1mM EDTA (mouse/clumpy cells only)

Fixing solution: deficient hRPMI
0.5% paraformaldehyde (1/8 of 4% stock)

Paraformaldeyhde (4%)

Paraformaldehyde is very toxic and aerates easily. Avoid breathing in the powder. Use a fume hood if necessary.

1. Mix required amount of paraformaldehyde (4g/100ml) to 2/3 final volume in ddH2O.
2. Heat to 60C while stirring in a fume hood (monitor temperature with thermometer).
3. Add 1 drop 2N NaOH to clear the solution.
4. Remove heat and add 1/3 vol 3x PBS.
5. Let cool and adjust to pH 7.2 with HCL.
6. Filter.