Title	FACs staining for intracellular protein
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Adapted from -	Gibco Protocol
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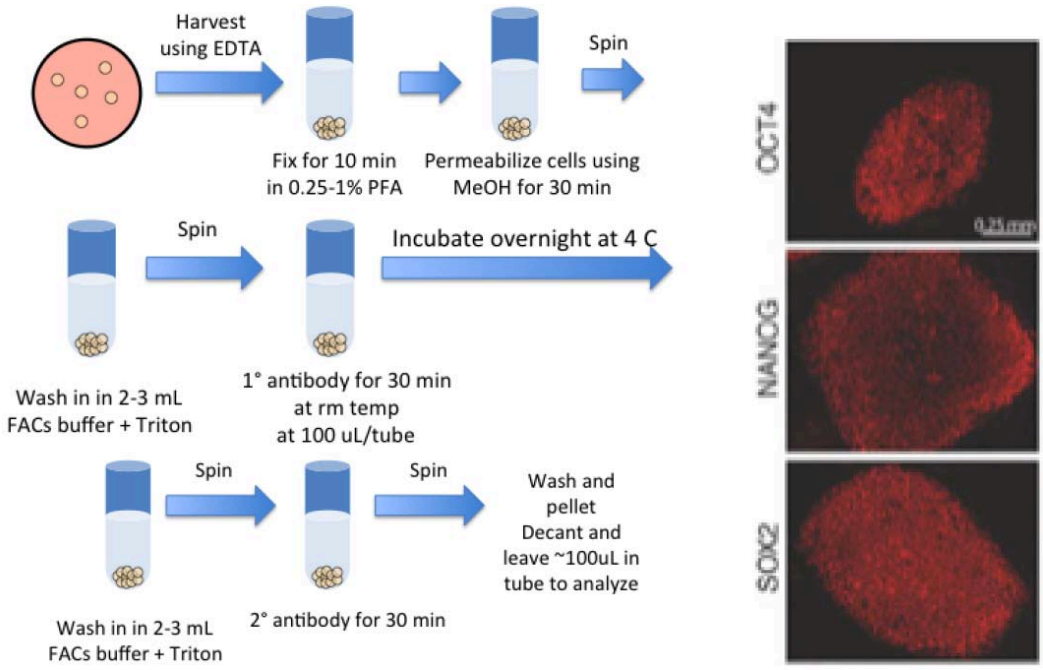
❖ Introduction:



Staining for intracellular OCT4, NANOG, and SOX2 for FACs analysis[1]

❖ Protocol:

A. Harvesting Undifferentiated Cells for FLOW

1. Add 1mL of trypsin/EDTA + 2% chick serum/well of a 6-well plate and incubate for 10 min at 37 C. (*For differentiated cells add 1mL of Dispase for 10 min, remove, add trypsin)
2. Scrape cells and pipette up/down to break up
3. Dilute with FACS buffer and filter the suspension through 100, 80 or 40 micron filter.
4. Spin down cells, 5 min, 1000 rpm
5. Pour off supernatant and add 1mL of PBS
6. Add paraformaldehyde so that the final concentration is 0.25-1% and fix for 10 min in 37 C water bath.
7. Spin down. Resuspend in 2mLs of FACS buffer. (Cells can be placed at 4 degrees for storage if needed)

8. Spin down cells and resuspend in 1mL of ice-cold 90% methanol. (Alternatively add 100% cold methanol to PBS + fixative to make 90% final concentration)
9. Incubate on ice for 30 min. (Proceed with staining or store cells at –20C in 90% Methanol)
10. Pellet cells.
11. Wash cells by adding 2-3mLs of FACS buffer + Triton. Pellet cells.
12. Pour off supernatant and add 100uL of pre-diluted primary Ab at 1:50.
13. Inc. overnight at 4 C
14. Wash cells by adding 2-3mLs of FACS buffer + Triton. Pellet cells.
15. Pour off supernatant and add 100uL of pre-diluted secondary Ab (1:500).
16. Incubate at RT for 30 min in the dark.
17. Wash and pellet cells. Pour off supernatant to leave ~100ul in tube (Cells can be diluted if needed). Transfer on ice to Flow lab to be analyzed.

❖ Materials:

- Begin with 5 x 10^5 – 1 x 10^6 cells/tube

Trypsin	FACS buffer
EDTA	Methanol
2% hick serum	Primary Ab
6-well tissue culture dish	Secondary Ab
PBS	Triton
0.25-1% PFA	

- FACS Buffer

PBS (w/o Ca/Mg++) + 2% FBS +0.1% NaN3
*0.5% BSA can be substituted for FBS

❖ Troubleshooting:

❖ References:

1. Dirk Hockemeyer, Frank Soldner, et al.Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. Nat. Biot. 27 851-857 (2009).

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