Title	FACs Surface Staining
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Adapted from -	Gibco Protocol
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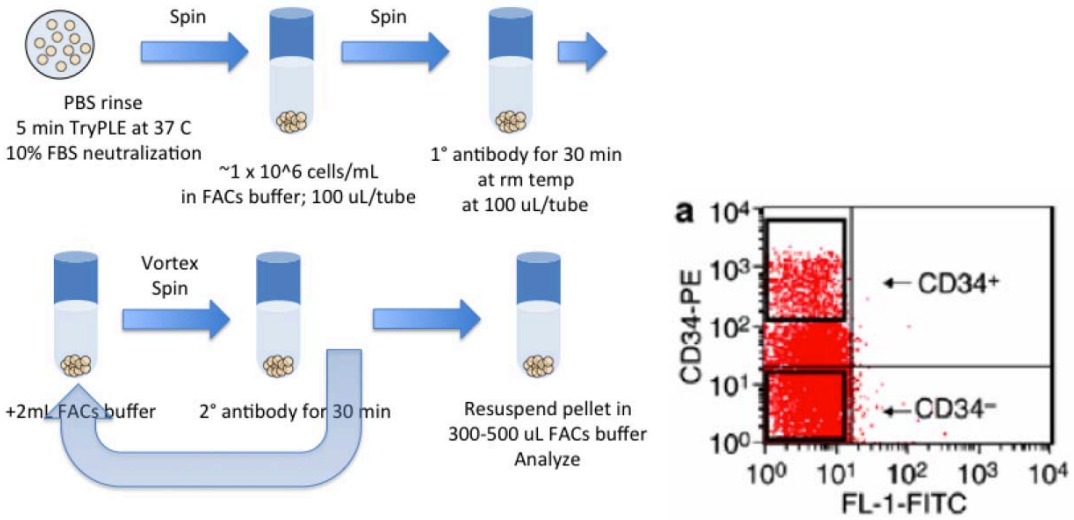
❖ Introduction:



Representative FACS dot plots and the gates used to isolate CD34+ and CD34- populations from low-density CML cells using an anti-CD34-PE antibody[1]

❖ Protocol:

1. PBS rinse cells
2. 5 minutes TryPLE at 37 C.
3. 10% FBS neutraliztion, spin at 1000 rpm for 5 minutes
4. Adjust cell density to ~1 x 10^6 cells/ml in FACs buffer and aliquot 100ul/tube
5. Spin down cells keep pellets
6. Dilute Abs in buffer to add 100ul/tube
7. Add primary Ab dilution to tube and vortex lightly
8. Incubate 30 min. at room temp OR 45 min. on ice

If direct staining (fluorochrome labeled Ab) work should be done in the dark. Follow steps 13-15

9. Add 2 ml FACs buffer to each tube, vortex, and spin down
10. Dump the supernatant, leaving ~ 100ul buffer with the pellet

11. Add 100ul of secondary Ab dilution
12. Vortex, and incubate 30 min. at room temp. or 45 min. on ice in the dark
13. Repeat step 9-10
14. Resuspend pellet in 300-500ul of FACs buffer
15. Transfer on ice for analysis

❖ Materials:

tryPLE
FBS
FACs buffer
Primary Ab
Secondary Ab

- FACS Buffer

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

❖ Troubleshooting:

❖ References:

1. X Jiang, Y Zhao, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. Leuk. 21 926-935 (2007).