FACSING FOR THE IL3 RECEPTOR
WITH DAVID DANKORT

Important note: if you wish to FACSort your cells make sure all reagents are sterile and all steps performed in a cell culture hood. Otherwise all steps can be carried out at your bench.

1. Wash cells in 1xPBS, trypsinize, inactivate trypsin with full media and spin down cells (1000rpm table top centrifuge). Wash cells once in 1xPBS and spin down.

2. Put cells on ice. Resuspend in 300ul PBS containing 2%FCS and 8ug/ml biotinylated anti-IL3 receptor [company name 5mg/ml stock concentration].

3. Incubate 1hr on ice.

4. Wash once in 10ml 1xPBS and spin down.

5. Wash once in 10ml 1xPBS containing 2% FCS and spin down.

6. Resuspend in 350-500ul PBS containing Streptavidin-conjugated FITC (or other flourophore) [16ul/ml]

At this point it is not necessary to wash but one could if they like.

6. FACS on FL1 (if FITC)

Appropriate controls include:
-ve cells that do not express the IL3R (eg fibroblasts)
-ve positive cells that are incubated with an isotype control primary antibody.
+ve cells that do express the receptor.