

Pullen Lab Protocol for Fc RI Probing of BMMC with a Countess FL II

This protocol is used to quickly phenotype bone marrow that has been skewed toward BMMC differentiation. For complete confirmation perform the same with the addition of a FITC anti-mouse CD117 (clone 2B8; BioLegend #105805), relevant isotype control (FITC Rat IgG2b, ; BioLegend #400633), and a GFP light cube.

Materials:

- BMMC at least four weeks in culture
- Countess FLII with an RFP light cube
- Purified anti-mouse CD16/32 (clone 93; BioLegend #101301)
- PE anti-mouse Fc RI (clone MAR-1; BioLegend #134307)
- PE Armenian hamster IgG isotype control (BioLegend #400907)
- Container with ice
- 2mL microcentrifuge tubes
- refrigerated microcentrifuge (set to 4°C)
- micropipettes with tips
- Incubation Buffer (0.5g BSA in 100mL 1X PBS)
- Blocking antibody solution (anti-CD16/32 diluted 1:50 in Incubation Buffer)

Procedure:

1. Obtain enough cells for about 5×10^5