

## FACS for Bone marrow macrophages

1. Remove cells from Valmark plates as usual. Wash x2 in FACS buffer: HBSS (no Ca, Mg, no phenol red) with 1% BSA, pH 7.4.
2. Pellet cells in eppendorf microfuge tube and resuspend  $10^6$  cells in 25  $\mu$ l FACS buffer containing 2 mg/ml human IgG.
3. Add 25  $\mu$ l of FACS buffer containing 10  $\mu$ g/ml M1/70, 5C6, M1/18, or your antibody. If you want the cells to be in  $Mn^{2+}$  buffer, use FACS buffer containing 200  $\mu$ M  $Mn^{2+}$ .
4. Incubate on ice for 30 min.
5. Wash x2 with FACS buffer
6. Resuspend  $10^6$  cells in 25  $\mu$ l FACS buffer containing 2 mg/ml human IgG.
7. Add appropriate amount of fluorescein- or Alexa 488- conjugated anti-rat IgG (for M1/70, 5C6, M1/18) or anti-mouse IgG for your antibody.
8. Alternatively, use  $F(ab')_2$  fragment of the  $e2^o$  antibody; this negates the requirement for human IgG block at this step.
9. Incubate on ice for 30 min.
10. Wash x2 with PBS or FACS buffer without BSA.
11. Either fix with paraformaldehyde or keep on ice and then run through flow cytometer.