Protocol adapted from those accompanying commercial markers18.

1. **Blocking (continuing from 9.7)**
   1. Resuspend the pellet in 98 μL of BSA buffer.
   2. Add 2 μL of FcR block.
   3. Incubate for 30 min at 4 °C.
   4. Add 1 mL of BSA buffer and centrifuge at 300 x *g* for 10 min at 4 °C.
   5. Aspirate supernatant.
2. **Antibody staining** 
   1. Resuspend the pellet in 90 μL of BSA buffer.
   2. Add 2 μL of FcR block.
   3. Add 1 μL of each antibody (Anti-CD31-APC, CD11b-VioBlue, Anti-ACSA-2-PE-Vio770, Anti-PSA-NCAM-PE, Anti-MBP) and mix well by pipetting up and down after each addition.
   4. Incubate for 10 min at 4 °C.
   5. Add 1 mL of BSA buffer and centrifuge at 300 x *g* for 10 min at 4 °C.
   6. Aspirate supernatant.
   7. Resuspend the pellet in 98 μL of BSA buffer.
   8. Add 2 μL of FcR block. Add 1 μL of FITC
   9. Incubate for 10 min at 4 °C.
   10. Add 1 mL of BSA buffer and centrifuge at 300 x *g* for 10 min at 4 °C.
   11. Aspirate supernatant.
   12. Proceed to 10.0 Fixation or 11.0 Flow Cytometry.

Biotec, M. *Cell surface flow cytometry staining protocol*, <<https://www.miltenyibiotec.com/US-en/applications/all-protocols/cell-surface-flow-cytometry-staining-protocol-pbs-bsa-1-50.html>> (2021).