



## Rabbit CD19+ Cell Depletion Protocol

### Materials

- Anti-Rabbit CD19 mouse IgG2a NullFc™ with site-specific biotinylation (Cobalt Biologics (Catalog # 200103)
- Dynabeads™ M-280 Streptavidin (Thermo Fisher Scientific catalog #11205D)  
**-OR-**
- Dynabeads™ Biotin Binder (Thermo Fisher Scientific catalog #11047)
- Magnetic support for Dynabeads™, such as DynaMag™-2 (Thermo Fisher Scientific catalog #12321D)
- Binding Buffer: Buffer: Ca<sup>2+</sup> and Mg<sup>2+</sup> free PBS supplemented with 0.1% bovine serum albumin (BSA) and 1 mM EDTA, pH 7.4

### Method

1. Rabbit PBMC's or similar cell preparation should be prepared by user prior to depletion
2. Wash Dynabeads™ as suggested by manufacturer. Use 50 µl of beads per depletion (starting from 3 x 10<sup>6</sup> cells or less, titrate as necessary)
3. Resuspend cells in Binding Buffer to 1 ml in a 15 ml conical tube
4. Add 20 µg of biotinylated anti-rabbit CD19, mix and incubate on ice for 10 minutes
5. Add 4 mls of Binding Buffer and centrifuge cells (500 x g for 5 minutes)
6. Aspirate liquid and resuspend cells in 500 µl Binding Buffer
7. Add 50 µl pre-washed beads and incubate at 4° C with gentle agitation (tube rotator, nutator or similar) for 30 minutes
8. Place the tube in the magnet for 2 min.
9. Transfer the supernatant containing the unbound cells to a new tube
10. Place the tube in the magnet for 2 min.
11. Transfer the supernatant containing the unbound cells to a new tube for further experiments

*This method should result in depletion of about 95% of the CD19+ cells. Additional round(s) of depletion may be performed to further reduce the CD19+ cells.*