SOP: **Isolation of primary mouse B (CD19$^+$) cells**
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Summary

For isolation of highly pure mouse CD19$^+$ B cells, the CD19$^+$ Miltenyi magnetic bead column purification kit is utilized (CliniMACS affinity-based technology, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany)

Materials List

1. C57BL/6 mice from Jackson labs
2. RPMI 1640, 1X, with 2mM L-glutamine (Cellgro, Cat# 10-040-CM)
3. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
4. 100 µm cell strainers (BD Biosciences, Cat# 352360)
5. 6 cm tissue culture dishes
6. 50mL Corning conical centrifuge tubes
7. 1mL syringes
8. Miltenyi CD19$^+$ Microbeads (Miltenyi, Cat# 130-052-201)
9. See Miltenyi product information sheets (below website) for necessary buffers and reagents
10. Eppendorf Refrigerated Centrifuge 5810R
11. Microscope

Medium (for primary cell suspension maintenance, on ice, before MACS separation)

RPMI, 1X
10% FBS

Procedure

1. All peripheral lymph nodes and spleens are collected from C57BL/6 mice from Jackson labs and pooled in a 6 cm dish containing RPMI 1640 supplemented with 10% FBS. Tissues and cell suspensions should be kept on ice at all times. Following removal of all media from the dish (retain this media as it contains some cells), the pooled lymph nodes and spleens are chopped into a fine pulp. The pulp is then scraped into a 100 µm cell strainer placed on a 50mL conical tube. Alternative rinsing cell strainer with RPMI 1640 supplemented with 10% FBS and mashing tissue through cell strainer with the back of a 1ml syringe plunger, until only white residue remains above strainer. Spin cells down and then resuspend in buffer as instructed in Miltenyi magnetic isolation protocol.

2. Follow procedure for CD19$^+$ Microbead kit (below website).

3. Set aside CD19$^+$ B cell fraction for freezing (or immediately freeze B cells during a break in isolation of other cells) as per freezing protocol (SOP: Cryopreservation of mouse naïve Tn, regulatory Tr, and B cells 03/04/2011 R.S. Hansen, T.K. Canfield (UW)).

4. It is recommended that purity of the isolation be tested by staining a small aliquot of purified cells for CD19 and analyzing by FACS. Purity should be >95% of cells in lymphocyte gate (non-debris).

Resources (datasheets for the above Miltenyi kit):

http://www.miltenyibiotec.com/download/datasheets_en/71/MiltenyiBiotec_DataSheet_CD19-MicroBeads,-mouse_130-052-201.pdf