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Dynal® Mouse B Cell Negative Isolation Kit

For research use only.

1. PRODUCT DESCRIPTION

1.1 Intended Use

Isolate untouched mouse B cells by depleting non-B cells (T cells, monocytes/macrophages, NK cells, dendritic cells, platelets, plasma cells, erythrocytes and granulocytes) from mouse spleen or lymph node cells. Other sources of mouse B cells can also be used as starting material after optimisation for the particular application. Isolated mouse B cells are bead- and antibody-free and are suitable for downstream applications.

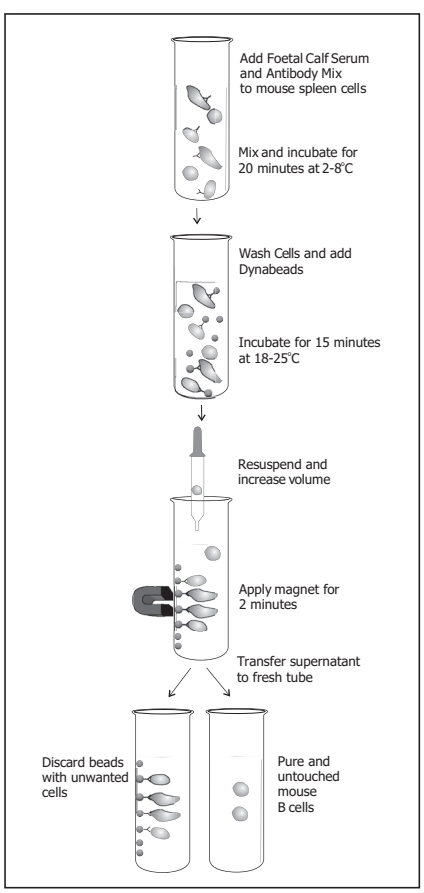

Fig. 1 Simple method for isolating untouched mouse B cells.

1.2 Principle of Isolation

Add a mixture of monoclonal antibodies against the non-B cells to the starting sample. Add Mouse Depletion Dynabeads to bind to the non-B cells during a short incubation. Separate the bead-bound cells by a magnet. Discard the bead-bound cells and use the remaining, untouched mouse B cells for any application (fig. 1).

1.3 Description of Materials

Dynal® Mouse B Cell Negative Isolation Kit contains uniform, superparamagnetic polystyrene Dynabeads® (4.5 µm diameter) coated with a polyclonal sheep anti-rat IgG antibody.

Materials Supplied

Dynal Mouse B Cell Negative Isolation Kit contains Mouse Depletion Dynabeads and Antibody Mix.

- **2 x 10 ml Mouse Depletion Dynabeads.**

  Supplied at 4 x 10^8 beads per ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide.
- **2 ml Antibody Mix.**

  The Antibody Mix contains a mixture of rat monoclonal antibodies against mouse CD43 (Ly-48), CD4 and Ter-119. CD43 is expressed on IL7-responsive pro-B cells, plasma cells and $CD5^+$ B cells (B-1 cells). These cells of the B lineage are thus removed during the isolation procedure, leaving resting, conventional B cells.
- **The kit will process up to 1 x 10^9 leucocytes.**

Additional Materials Required

- Magnet (Dynal MPC™): See www.invitrogen.com/magnets-selection for magnet recommendations.
- Heat inactivated Fetal Calf Serum (FCS).
- Mixer allowing both tilting and rotation.
- Buffer 1: PBS w/0.1% BSA and 2 mM EDTA, pH 7.4.
- Buffer 2: RPMI-1640 w/10% FCS.

Keep the buffers cold!

Important Notes:

BSA can be replaced by FCS.

EDTA can be replaced by sodium citrate.

PBS containing Ca^{2+} or Mg^{2+} is not recommended.

2. PROTOCOLS

2.1 Dynabeads Washing Procedure

Dynabeads should be washed before use.

1. Resuspend the Dynabeads in the vial.
2. Transfer the desired volume of Dynabeads to a tube.
3. Add the same volume of Buffer 1, or at least 1 ml, and mix.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed Dynabeads in the same volume of Buffer 1 as the initial volume of Dynabeads (step 2).

2.2 Sample Preparation

Please visit www.invitrogen.com/cell-isolation and follow our QuickLinks for recommended sample preparation procedures.

2.3 Critical Steps for Cell Isolation

- Use a mixer that provides tilting and rotation of the tubes to ensure Dynabeads do not settle at the bottom of the tube.
- Never use less than 200 µl Dynabeads per 1 x 10^7 leucocytes.
- It is critical to follow the magnet recommendations to ensure a successful isolation.

2.4 Isolation of Mouse B Cells from Spleen or Lymph Node Leucocytes

This protocol is based on 1 x 10^7 leucocytes. It is scalable from 1 x 10^7-1 x 10^9 cells, (see table 1).

1. Transfer 100 µl (1 x 10^7) leucocytes in Buffer 1 to a tube.
2. Add 20 µl heat inactivated FCS.
3. Add 20 µl of Antibody Mix.
4. Mix well and incubate for 20 min at 2-8°C.
5. Wash the cells by adding 2 ml Buffer 1. Mix well by tilting the tube several times and centrifuge at 300 x g for 8 min at 2-8°C. Discard the supernatant.
6. Resuspend the cells in 800 µl Buffer 1.
7. Add 200 µl pre-washed Mouse Depletion Dynabeads.
8. Incubate for 15 min at 18-25°C with gentle tilting and rotation.
9. Resuspend the bead-bound cells by **gently** pipetting 5 times using a pipette with a narrow tip opening, (e.g. a 1000 µl pipette tip or a 5 ml serological pipette).
10. Add 1 ml Buffer 1.
11. Place the tube in the magnet for 2 min.
12. Transfer the supernatant to a new tube.

The supernatant contains the negatively isolated mouse B cells.

Table 1. Volume requirements for mouse B cell isolation per 1 x 10^7 starting leucocytes.

	Working volume per 1 x 10^7 leucocytes
Cell volume (step 1)	100 µl
FCS (step 2)	20 µl
Antibody Mix (step 3)	20 µl
Washing (step 5)	2 ml
Resuspension (step 6)	800 µl
Mouse Depletion Dynabeads (step 7)	200 µl
Volume added before magnet (step 10)	1 ml
Dynal MPC recommended	MPC-L/MPC-15/MPC-50

When working with higher cell numbers, scale up all reagents and volumes accordingly. Up to 5 x 10^7 leucocytes can be processed in a single 15 ml tube. Up to 2 x 10^8 leucocytes can be processed in a single 50 ml tube.

2.5 Downstream Applications

Isolated mouse B cells can be used in applications such as flow cytometry, functional assays and studies on B cell activation, proliferation and differentiation.

3. GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

Storage/Stability

This kit is stable until the expiry date stated on the label when stored unopened at 2-8°C.

Store opened vials at 2-8°C and avoid bacterial contamination.

Keep Dynabeads in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend well before use.

Technical Support

Please contact Invitrogen Dynal for further technical information (see contact details).

Warning And Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated.

Follow appropriate laboratory guidelines.

This product contains 0.02% sodium azide as a preservative, which is cytotoxic. **Avoid pipetting by mouth!** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build up.

Certificate of Analysis (CoA) is available upon request.

Material Safety Data Sheet (MSDS) is available at http://www.invitrogen.com.

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