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Cat. no. 113.13D

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Dynal® B Cell Negative Isolation Kit

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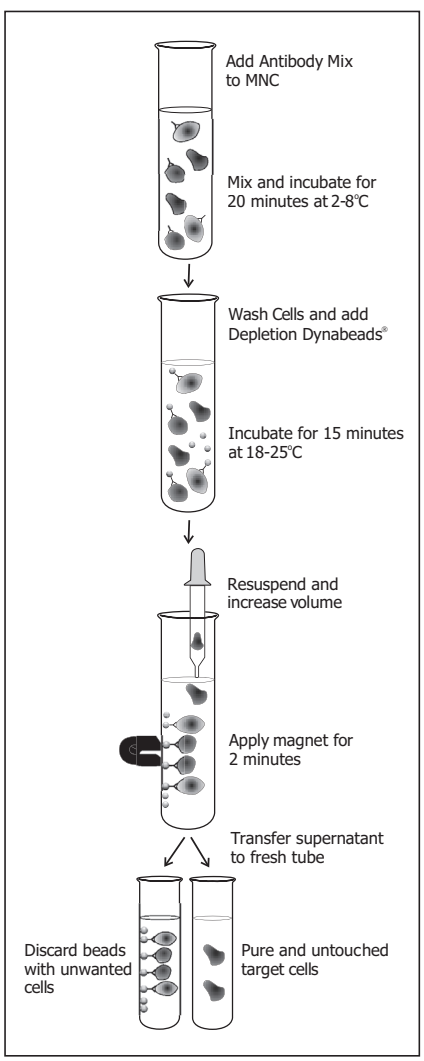

Fig. 1 Simple method for isolating untouched human B cells.

1. PRODUCT DESCRIPTION

1.1 Intended Use

Isolate untouched human naïve B cells by depleting activated B cells, T cells, NK cells, monocytes, macrophages, granulocytes, platelets, plasma cells and erythrocytes from peripheral blood. Isolated B cells are bead- and antibody-free and are suitable for any downstream application.

This kit contains anti-CD43 antibodies. CD43 is expressed on B cells from various lymphomas (e.g. CLL, ALL) as well as subsets of activated B cells.

1.2 Principle of Isolation

Add a mixture of monoclonal antibodies against the non-B cells to the starting sample. Add 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 human B cells for any application (fig.1).

1.3 Materials Supplied

Dynal® B Cell Negative Isolation Kit for untouched human cells contains Depletion Dynabeads and Antibody Mix.

- **2 x 5 ml Depletion Dynabeads**
  Supplied in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide.
- **1 ml Antibody Mix**
  The antibody mix contains mouse IgG antibodies against CD2, CD14, CD16 (specific for CD16a and CD16b), CD36, CD43 and CD235a (Glycophorin A).

  Supplied in PBS with 0.1% BSA and 0.02% sodium azide (NaN_3).
- **The kit will process up to 5 x 10^8 cells.**

1.4 Additional Materials Required

- Magnet: See www.invitrogen.com/magnets-selection for magnet recommendations.
- Mixer allowing both tilting and rotation.
- Isolation buffer: Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) (e.g. Gibco cat. no. 14190-094) supplemented with 0.1% BSA and 2mM EDTA.
- Lymphoprep™ for MNC preparation (Axis Shield PoC, Norway, www.axis-shield-poc.com).

Keep the buffers cold!

Note:

BSA can be replaced by human serum albumin (HSA) or 2% FBS/FCS.

EDTA can be replaced by sodium citrate.

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

2. PROTOCOL

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, 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 as the initial volume of Dynabeads (step 2).

2.2 Sample Preparation

Prepare MNC from buffy coat. Please visit www.invitrogen.com/cellisolation 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 100 µl Dynabeads per 1 x 10^7 MNC sample.
- Dynal MPC™-1 (Cat. no. 120.01D) should not be used with this product.

2.4 Isolation of Human B Cells from MNC

This protocol is based on 1 x 10^7 MNC. It is scalable from 1 x 10^7-5 x 10^8 cells, (see table 1).

1. Transfer 100 µl (1 x 10^7) MNC in Buffer to a tube.
2. Add 20 µl of Antibody Mix.
3. Mix well and incubate for 20 min. at 2-8°C.
4. Wash the cells by adding 2 ml Buffer. Mix well by tilting the tube several times and centrifuge at 300 x g for 8 min at 2-8°C. Discard the supernatant.
5. Resuspend the cells in 800 µl Buffer.
6. Add 200 µl pre-washed Depletion Dynabeads.
7. Incubate for 15 min at 18-25°C with gentle tilting and rotation.
8. 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).
9. Add 1 ml Buffer and mix.
10. Place the tube in the magnet for 2 min.
11. Transfer the supernatant containing the negatively isolated B cells to a new tube.
12. Repeat steps 9-11 and pool the two supernatants.

Table 1. Volumes for human B cell isolation (1 x 10^7 to 5 x 10^8 MNC)

2.5 Downstream Applications

	Working volume per 1 x 10^7 MNC
Cell volume (step 1)	100 µl
Antibody Mix (step 2)	20 µl
Washing (step 4)	2 ml (Max. 40 ml when scaled up)
Resuspension (step 5)	800 µl (Max. 5 ml when scaled up)
Depletion Dynabeads (step 6)	200 µl
Increase volume (step 9)	1 ml (Max. 35 ml when scaled up)

Isolated B cells can be used in applications such as:

- Flow cytometry (for recommended products and protocols visit www.invitrogen.com/immunology.)
- Antigen presentation by B cells and interaction with other cells of the immune system.
- Analysis of B cell immunoglobulin class switching and somatic hypermutation.
- Analysis of B cell activation, proliferation and differentiation.
- B cell signalling pathway studies.

3. GENERAL INFORMATION

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

Description of Materials

Depletion Dynabeads are uniform, superparamagnetic polymer beads (4.5 µm diameter) coated with a monoclonal human anti-mouse IgG antibody. The antibody coated onto Depletion Dynabeads recognises all mouse IgG subclasses and is Fc-specific. The antibody on the Dynabeads is a human IgG4 anti-mouse IgG. The source of the human monoclonal antibody is free of

Human Immunodeficiency Virus (HIV), Hepatitis-B Virus (HBV) and Hepatitis-C Virus (HCV).

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.

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/Compliance is available upon request.

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

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4. REFERENCES

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Contact details for your local Invitrogen sales office/technical support can be found at http://www.invitrogen.com/contact

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