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invitrogen bead separations

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Dynabeads® CD15

For research use only

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1. PRODUCT DESCRIPTION

1.1 Intended Use

Isolate or deplete $CD15^+$ cells directly from whole blood, buffy coat or MNC suspensions with Dynabeads CD15. For rapid and consistent results in protein or gene expression analysis, lyse the granulocytes while they are still attached to the beads and directly process for further molecular analysis.

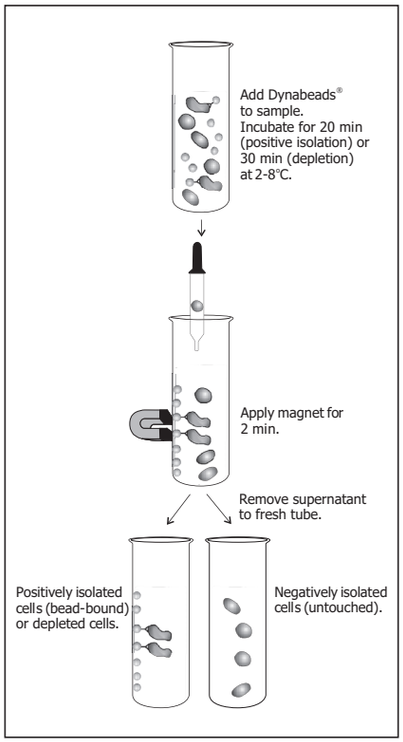

Fig. 1 Overview of method

1.2 Principle of Isolation

Dynabeads are mixed with the cell sample in a tube. The Dynabeads will bind to the target cells during a short incubation, and then the bead-bound cells are separated by a magnet.

- **Positive isolation** – discard the supernatant and use the bead-bound cells for downstream applications (e.g. molecular analysis).
- **Depletion** – discard the bead-bound cells and use the remaining, untouched cells for any application.

1.3 Description of Materials

Dynabeads CD15 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a monoclonal mouse IgM antibody recognizing the CD15 membrane antigen. The CD15 antigen is predominantly expressed on human neutrophil and eosinophil granulocytes, and to a varying degree on monocytes (1). The CD15 antigen shows heterogeneous expression on normal myeloid precursor cells, myeloid leukemias, myeloid cell lines and Sternberg-Reed cells. The CD15 antigen is widely distributed outside the hematopoietic system.

Materials Supplied

- **5 ml Dynabeads CD15**
  4 x 10^8 beads/ml in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide (NaN_3).
- This product will process up to 2 x 10^9 cells

Additional Materials Required

Materials that are not included, but are needed to perform the entire protocol:

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

Important Notes:

- BSA can be replaced by human serum albumin (HSA) or Fetal Calf Serum (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 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 - Whole Blood and Buffy Coat

Blood and buffy coat can be diluted before use to decrease the granulocyte concentration. This is to ensure that the number of Dynabeads per target cell is higher than 4:1 in granulocyte-rich samples.

1. Dilute whole blood (1+1) in Buffer 1
2. Dilute buffy coat (1+4) in Buffer 1

Dynabeads can be added directly to undiluted blood or buffy coat if reduced cell isolation efficiency is tolerated (e.g. rapid isolation for molecular studies).

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.
- When incubating Dynabeads and cells, the incubation temperature must be 2-8°C to reduce phagocytic activity and other metabolic processes.
- Never use less than 25 µl (1 x 10^7) Dynabeads per ml of cell sample and at least 4 Dynabeads per target cell.

Table 1: Volume of Dynabeads added per ml of cell sample. The volumes can be scaled up as required.

	Positive isolation	**Depletion**
Sample volume - 1 x 10^7 MNC*/ml - diluted whole blood - diluted buffy coat	1 ml	1 ml
Volume of Dynabeads	25 µl	50 µl
Total no. of cells processed per product	2 x 10^9 cells	1 x 10^9 cells

* if the concentration of MNC is increased, the volume of Dynabeads must be increased accordingly. Cell concentration can be up to 1 x 10^8 cells per ml.

2.4 Depletion or Positive Isolation of $CD15^+$ Cells

1. Add the appropriate volume of Dynabeads to the prepared sample according to table 1.
2. Incubate for 20 min (positive isolation) or 30 min (depletion) at 2 - 8°C with gentle tilting and rotation.
3. Place the tube in a magnet for 2 min.
4. For depletion, transfer supernatant to a new tube for further use.
5. For positive isolation, discard the supernatant and wash the bead-bound cells 3 times by resuspending in Buffer 1 to the original sample volume, and separate using a magnet.

For molecular studies, lyse cells while still attached to the beads and transfer supernatant to a new tube for protein or gene expression analysis.

3. GENERAL INFORMATION

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

Storage/Stability

This product 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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5.8. Warranty

The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Invitrogen Dynal's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen Dynal's expense, of any products which shall be defective in manufacture, and which shall be returned to Invitrogen Dynal, transportation prepaid, or at Invitrogen Dynal's option, refund of the purchase price.

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4. REFERENCES

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4 Smith D (2004) Liver Transplant-Associated Graft-versus-host Disease. ASHI Quarterly Q1: 12-13.

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