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invitrogen bead separations

Cat. no. 111.45D

Rev. no. 003

Dynabeads® CD4

For research use only.

1. PRODUCT DESCRIPTION

1.1 Intended Use

Isolate or deplete human $CD4^+$ T cells directly from whole blood, buffy coat or MNC with Dynabeads CD4. For rapid and consistent results in protein or gene expression analysis, lyse the $CD4^+$ T cells while they are still attached to the beads and directly process for further molecular analysis. For downstream cell-based applications (requiring bead-free $CD4^+$ cells), Invitrogen Dynal recommends the Dynabeads® FlowComp™ Human CD4 kit (cat. no. 113.61D).

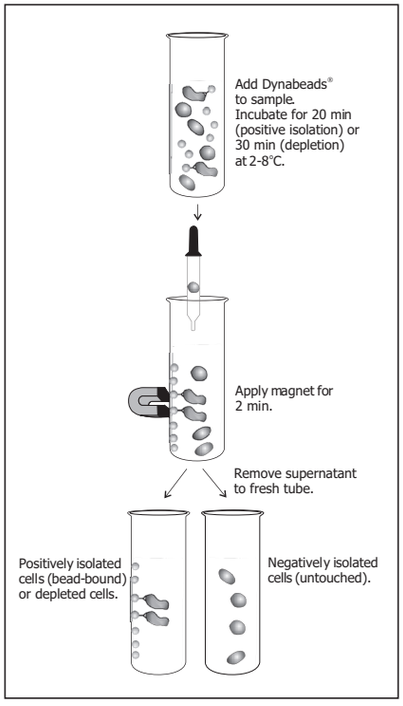

Fig. 1 Overview of method

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 molecular applications.
- **Depletion** – discard the bead-bound cells and use the remaining, untouched cells for any application.

1.3 Description of Materials

Dynabeads CD4 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a primary monoclonal antibody specific for the CD4 membrane antigen on human cells.

The primary CD4 antibody is coupled to the Dynabeads via a secondary human IgG4 anti-mouse IgG antibody. The source of the human monoclonal antibody is free of Human Immunodeficiency Virus (HIV), Hepatitis-B Virus (HBV) and Hepatitis-C Virus (HCV).

Materials Supplied

- **5 ml Dynabeads CD4.**

  4×10^8 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 2×10^9 cells.**

Additional Materials Needed

- 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. 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

Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC or tissue digests.

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

Whole Blood and Buffy Coat

Most depletions and positive isolations can use whole blood and buffy coat as a starting sample. Buffy coat is 8-10 times more concentrated than whole blood with regard to number of leucocytes. For this product you have to wash the blood/buffy coat to remove interfering soluble factors.

1. Dilute the whole blood or buffy coat in Buffer 1 (1+2).
2. Centrifuge at 600 x g for 10 min at 2-8°C.
3. Discard the plasma fraction/upper layer. Resuspend blood to the original volume in Buffer 1 and buffy coat 1+1 in Buffer 1 before adding the beads.

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 Dynabeads per ml of cell sample.

Table 1: Volume of Dynabeads added per 10^7 cells. The volumes can be scaled up as required.

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

2.4 Depletion or Positive Isolation of $CD4^+$ T 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 1 min. Never use less than 1 ml Buffer 1 in each washing step.

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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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.

Claims for merchandise damaged in transit must be submitted to the carrier.

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4. REFERENCES

1. Roscic-Mrkic B et al (2003) RANTES (CCL5) uses the proteoglycan CD44 as an auxiliary receptor to mediate cellular activation signals and HIV-1 enhancement. Blood 102: 1169-1177.
2. Peng Koh K et al (2004) T cell-mediated vascular dysfunction of human allografts results from IFN-g dysregulation of NO synthase. J. Clin. Investigation. 114:846-856.
3. Dai KZ et al (2004) Transcriptional activation of the SH2D2A gene is dependent on a cyclic adenosine 5'-monophosphate-responsive element in the proximal SH2D2A promoter. J. Immunol. 172: 6144-6151.

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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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