DETACHaBEAD® CD19

For research use only

This product is intended for detachment of Dynabeads® CD19 Pan B from CD19+ cells.

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1. PRODUCT DESCRIPTION

1.1. Intended Use

DETACHaBEAD CD19 is intended for detachment of Dynabeads® CD19+ cells after isolation with Dynabeads CD19 Pan B, cat. no. 111.43D (not supplied).

1.2. Principle of Detachment

DETACHaBEAD CD19 is added to the bead-bound cells after isolation with Dynabeads CD19 Pan B. The DETACHaBEAD CD19 reagent will detach the Dynabeads and primary antibody from the CD19+ cells. Dynabeads are subsequently removed using a Dynal magnet, and the detached CD19+ cells are ready for any downstream application.

1.3. Description of Materials

DETACHaBEAD CD19 contains polyclonal anti-Fab antibodies specific for the CD19 antibody on the Dynabeads CD19 Pan B. No cross-reaction with human IgG, IgM or IgA has been detected in qualitative ELISA tests.

Materials Supplied

• 5 ml DETACHaBEAD CD19 in 0.15 M phosphate buffered saline (PBS), pH 7.4.

Note: Due to the high protein concentration it is not unusual to see some precipitation in the DETACHaBEAD CD19 component. This will not influence on product performance or quality.

Additional Materials Required

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

• Dynabeads CD19 Pan B (cat. no. 111.43D) for positive isolation of CD19+ cells.
• Magnet: See www.invitrogen.com/magnets-selection for magnet recommendations.
• Mixer allowing both tilting and rotation.
• Tubes: We recommend adapting the tube size to the working volume (e.g. using 1.5 ml Eppendorf tubes for small volumes).
• Medium, e.g. RPMI 1640 w/1% Foetal Bovine Serum (FBS).

Table 1: Volumes of Dynabeads CD19 Pan B, medium and DETACHaBEAD CD19.

<table>
<thead>
<tr>
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<th>Volumes</th>
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<tbody>
<tr>
<td>Volume Dynabeads CD19 Pan B used for cell isolation</td>
<td>25 µl</td>
</tr>
<tr>
<td>Volume medium to resuspend bead-bounds cells (step 2)</td>
<td>250 µl</td>
</tr>
<tr>
<td>Volume DETACHaBEAD CD19 (step 3)</td>
<td>10 µl</td>
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</table>

1. Start with bead-bound cells after isolation with Dynabeads CD19 Pan B as recommended (i.e. use 25 µl of Dynabeads CD19 Pan B).
2. Resuspend the bead-bound cells in 250 µl medium (never use less than 100 µl medium).
3. Add 10 µl DETACHaBEAD CD19 per 25 µl Dynabeads used for cell isolation (never use less than 10 µl DETACHaBEAD CD19). 4. Incubate for 45 min at room temperature with gentle mixing.

5. To enhance the release of cells, pipette the solution a few times before placing the tube in the magnet for approx. 1 min.
6. Transfer the supernatant containing the released cells to a new tube. To obtain residual cells, wash the beads 2-3 times in 500 µl medium and pool the supernatants.
7. Wash the detached cells thoroughly by resuspending the cells in a total volume of 10 ml medium and centrifuge for 6 min at 400 x g to remove any residual DETACHaBEAD reagent.
8. Resuspend the cells in medium and use in any downstream application.

The isolated CD19+ cells are pure, viable and are free from antibody or beads bound to the surface.

2. PROTOCOL

The following protocol is recommended for a starting sample of 2.5 x 10^7 MNC in 1 ml.

The protocol is scalable, but a minimum detach volume (medium) and DETACHaBEAD is required (see below).

3. GENERAL INFORMATION


Storage/Stability

This product is stable until the expiry date stated on the label when stored unopened at 2-8°C. DO NOT FREEZE. Store opened vials at 2-8°C and avoid bacterial contamination.

Technical Support

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

Warning And Limitations

This product is for research use only. Not for human or animal therapeutic or diagnostic use.

Certificate of Analysis (CoA) is available upon request.

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

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Note: To avoid cell loss when working with small volumes, we recommend to keep the suspension in the bottom of the tube while still providing good mixing.
Warranty
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4. REFERENCES