Dynal® T Cell Negative Isolation Kit

For research use only.

This kit depletes activated T cells.

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1. KIT CONTENTS

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<thead>
<tr>
<th>Dynal T Cell Negative Isolation Kit</th>
<th>Cat. no. 113.11D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells processed: Up to 5 x 10^8</td>
<td></td>
</tr>
<tr>
<td>Depletion Dynabeads*</td>
<td>5 ml</td>
</tr>
<tr>
<td>Antibody Mix</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

* Supplied in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide (NaN₃).

2. PRODUCT DESCRIPTION

Intended Use

This product is intended for isolation of untouched human T cells by depleting non-T cells (B cells, NK cells, monocytes, platelets, dendritic cells, granulocytes, erythrocytes) and activated T cells from peripheral blood mononuclear cells (PBMC). Isolated T cells are bead- and antibody-free and are suitable for any downstream application.

3. PROTOCOL

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 Isolation 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 Isolation Buffer as the initial volume of Dynabeads (step 2).

Preparations

Prepare a PBMC suspension (low platelet numbers, see Technical Recommendations). Resuspend the suspension at 1 x 10^8 PBMC per ml in Isolation Buffer.

Isolation Procedure

This protocol is based on 1 x 10^7 PBMC and is scalable from 1 x 10^7 - 5 x 10^8 cells according to table 1.

<table>
<thead>
<tr>
<th>Working volume per 1 x 10^7 PBMC</th>
<th>Working volume per 5 x 10^8 PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell volume (step 1)</td>
<td>100 µl</td>
</tr>
<tr>
<td>PBS/FCS (step 2)</td>
<td>20 µl</td>
</tr>
<tr>
<td>Antibody Mix (step 3)</td>
<td>20 µl</td>
</tr>
<tr>
<td>Washing (step 5)*</td>
<td>2 ml</td>
</tr>
<tr>
<td>Resuspension (step 6)*</td>
<td>900 µl</td>
</tr>
<tr>
<td>Depletion Dynabeads (step 7)</td>
<td>100 µl</td>
</tr>
<tr>
<td>Volume added before magnet separation (step 10)*</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

* Note that the difference in volumes for small vs. large scale protocol is based on getting good working volumes during incubation with beads. A shift from small to large scale parameters may be done at any level of cells processed, as long as the resulting volume during incubation with beads is appropriate for the tubes used (more than 1/5 of total tube volume).

1. Transfer 100 µl (1 x 10^7) PBMC in Isolation Buffer to a tube.
2. Add 20 µl heat inactivated FCS.
3. Add 20 µl Antibody Mix.
4. Mix well and incubate for 20 min at 2-8°C.
5. Wash the cells by adding 2 ml Isolation 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.
6. Resuspend the cells in 900 µl Isolation Buffer.
7. Add 100 µl pre-washed 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 Isolation Buffer.
11. Place the tube in the magnet for 2 min.
12. Transfer the supernatant to a new tube.
13. Repeat step 10-12.

The supernatant contains the negatively isolated human T cells.
4. GENERAL INFORMATION
Manufactured by Invitrogen Dynal AS.

Description of Materials
Dynabeads are uniform, superparamagnetic polymer beads (4.5 μm diameter) coated with a monoclonal human anti-mouse IgG antibody. The antibody coated onto Dynabeads recognizes 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).
The Antibody Mix contains mouse IgG antibodies for CD14, CD16 (specific for CD16a and CD16b), HLA Class II DR/DP, CD56 and CD235a (Glycophorin A).

Storage and 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).

Warnings 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/Compliance is available upon request.

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5. TECHNICAL RECOMMENDATIONS
Sample Preparation
Preparation of PBMC from Buffy Coat to Obtain Low Platelet Numbers:
1. Dilute 10 - 18 ml buffy coat with PBS (without Ca2+ and Mg2+) pH 7.4., to a total volume of 35 ml at 18-25°C (RT).
2. Add the diluted buffy coat on top of 15 ml of Lymphoprep.
3. Centrifuge at 160 x g for 20 min at RT. Allow to decelerate without brakes.
4. Remove 20 ml of supernatant to eliminate platelets.
5. Centrifuge at 350 x g for 20 min at RT. Allow to decelerate without brakes.
6. Recover PBMC from the plasma/Lymphoprep interface and transfer the cells to a 50 ml tube.
7. Wash PBMC once with Isolation Buffer by centrifugation at 400 x g for 8 min at 2-8°C.
8. Wash PBMC twice with Isolation Buffer by centrifugation at 225 x g for 8 min at 2-8°C and resuspend the PBMC at 1 x 10^6 PBMC per ml in Isolation Buffer.

6. 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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