

Dynabeads[®] Regulatory CD4⁺CD25⁺ T Cell Kit

For research use only.

1. KIT CONTENTS
2. PRODUCT DESCRIPTION
3. PROTOCOL
4. GENERAL INFORMATION
5. TECHNICAL SUPPORT

1. KIT CONTENTS

Dynabeads [®] Regulatory CD4 ⁺ CD25 ⁺ T Cell Kit	
Catalog no.	113.63D
Number of cells (MNC) processed: 1x10 ⁹	
Antibody Mix Human CD4 (Regulatory T Cell Kit)	2 x 1 ml
Depletion MyOne™ Dynabeads [®] (Regulatory T Cell Kit)	2 x 5 ml
Dynabeads [®] CD25 (Regulatory T Cell Kit)	1 x 5 ml
DETACHaBEAD [®] (Regulatory T Cell Kit)	1 x 2 ml

2. PRODUCT DESCRIPTION

This product is intended for magnetic isolation of CD4⁺CD25⁺ regulatory T cells from human mononuclear cells (MNCs). The CD4⁺CD25⁻ cell fraction can be used as effector cells in downstream inhibitory assays. The supplied protocol describes magnetic labeling and isolation from 1 x 10⁸ MNCs. In the first step the non-CD4⁺ MNC are labelled with Antibody Mix Human CD4. In the second step Depletion MyOne Dynabeads are added to remove the non-CD4⁺ MNCs. In the third step Dynabeads CD25 are added to the CD4⁺ T cells to capture the CD4⁺CD25⁺ T cells and in the last step Dynabeads CD25 are removed from the cells.

Downstream Applications

Isolated cells may be used directly in any downstream application including flow cytometry and cell expansion protocols.

For recommended products and protocols visit www.invitrogen.com/immunology.

Additional requirements

- Isolation buffer: Ca²⁺ and Mg²⁺ free phosphate buffered saline (PBS) (f.ex. Gibco cat.no. 14190-094) supplemented with 0.1% BSA and 2mM EDTA (see Technical Recommendations for further information).
- Foetal Bovine Serum (FBS)
- Media: RPMI or equivalent w.1% FBS
- Mixer allowing both tilting and rotation.
- Magnet (Dyna[®] MPC™): See www.invitrogen.com/magnets-selection for magnet recommendations.

- Flow cytometry antibody reagents (optional): see www.invitrogen.com/immunology for recommended products and protocols.

▲ Critical notes

- Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads do not settle at the bottom of the tube.
- This product should not be used with the Dynal MPC-1 magnet (Cat. No. 120.01D)
- Never use less than recommended volume of Dynabeads.
- Carefully follow the recommended pipetting volumes and incubation times.
- Avoid air bubbles during pipetting.
- Use primary fluorescent conjugated antibodies for flow cytometry. Avoid using secondary antibodies specific for mouse antibodies for flow cytometry staining.

3. PROTOCOL

Approximately 3-10% of the CD4⁺ T cell population expresses the CD25 antigen. This kit isolates highly pure CD4⁺ CD25⁺ regulatory T cells that express the intracellular transcription factor FOXP3. This protocol describes magnetic labelling and isolation of CD4⁺CD25⁺ regulatory T cells from 1x10⁸ human mononuclear cells (MNCs) using Dynabeads Regulatory CD4⁺CD25⁺ T Cell Kit. For scale-up to larger cell numbers, adjust the volumes accordingly.

Preparations

- Isolate MNCs from anti-coagulated peripheral blood or leukocyte enriched buffy coat using standard procedure (see Technical Recommendations for further information).
- Prepare isolation buffer and RPMI w. 1% FBS.

Isolation procedure

1. Resuspend 1 x 10⁸ MNCs in 500 µl isolation buffer and add 200 µl FBS and 200 µl Antibody Mix Human CD4. Mix well and incubate for 20 min at 2 – 8°C.
2. Add 10 ml cold isolation buffer to wash cells, followed by centrifugation for 8 min at 350xg.
3. Remove and discard the supernatant.
4. Add 2 ml cold isolation buffer to the cell pellet and resuspend.
5. Add 1 ml resuspended Depletion MyOne Dynabeads and mix well. Incubate for 15 min at room temperature under rolling and tilting.
6. Resuspend the bead-bound cells by **vigorously** pipetting 5 times using a pipette with a narrow tip opening (e.g. a 1000 µl pipette tip or a 5 ml serological pipette).
7. Add 3 ml of isolation buffer.
8. Place the tube in the magnet for 3 min.
9. Transfer the supernatant containing the bead-free CD4⁺ T cells to a new tube.
10. Repeat step 8–9 with the tube containing the supernatant to remove residual Depletion MyOne Dynabeads.
11. Spin down the cells for 8 min at 350xg and resuspend the cells in isolation buffer to a concentration of 1.5 x 10⁷ CD4⁺ cells/ml
12. Add 200 µl Dynabeads CD25 per 1,5 x 10⁷ CD4⁺ cells.
13. Mix well and incubate for 25 min at 2 – 8°C with rolling and tilting.
14. Place the tube in the magnet for a minimum of 1 minute. Carefully remove the supernatant containing the CD4⁺CD25⁻ (effector) cells.
15. Remove the tube from the magnet and **carefully** resuspend the bead-bound cells in 5 ml isolation buffer by gently shaking the tube instead of pipetting the cells.
16. Place the tube in the magnet for a minimum of 1 min. Remove and discard the supernatant.
17. Wash one more time by **carefully** resuspending the cells in 5 ml isolation buffer and gently shaking the tube.
18. Place the tube in the magnet for a minimum of 1 minute. Remove and discard the supernatant.
19. Resuspend the bead-bound cells in 500µl RPMI w.1% FBS
20. Add 80 µl DETACHaBEAD and incubate for 45 minutes at room temperature with tilting and rotation.
21. Place the tube in the magnet for a minimum of 1 minute. Carefully remove the supernatant containing the CD4⁺CD25⁺ cells to a new tube.
22. Wash the Dynabeads CD25 twice in 1 ml RPMI w.1% FBS to obtain the residual cells and collect the supernatant after separation on a magnet.
23. Add 10 ml RPMI w.1% FBS to wash the cells, followed by centrifugation for 8 min at 350xg. Perform this washing step twice.
24. Discard the supernatant and resuspend the cells in a preferred cell culture medium.

For further technical advice please visit www.invitrogen.com/cellisolation.

4. GENERAL INFORMATION

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

Description of Materials

Depletion MyOne Dynabeads are uniform, superparamagnetic polystyrene beads (1 µm diameter). Dynabeads CD25 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter).

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.

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 (CoA) is available upon request.

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

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5. TECHNICAL SUPPORT

General troubleshooting

- To avoid aggregation of cells it is recommended to use gamma-globulin or Fc blocking reagents.

Isolation buffer

PBS (phosphate buffered saline) from Gibco (cat.no. 14190-094) supplemented with 0.1% BSA and 2mM EDTA.

If preferred, PBS with 2% fetal calf serum and 1 mM EDTA may be used.

Preparation of MNC from Buffy Coat

This method gives low platelet numbers and is recommended for use with the Dynabeads® Regulatory CD4⁺CD25⁺ T Cell Kit.

1. Dilute 10–18 ml buffy coat with PBS with 0.1% BSA + 0.6% Na-citrate or 2 mM EDTA to a total volume of 35 ml at 18–25°C.
2. Add the diluted buffy coat on top of 15 ml of Lymphoprep™.
3. Centrifuge at 160 x g for 20 min at 20°C. Allow to decelerate without brakes.
4. Remove 20 ml of supernatant to eliminate platelets.
5. Centrifuge at 350 x g for 20 min at 20°C. Allow to decelerate without brakes.
6. Recover MNC from the plasma/Lymphoprep interface and transfer the cells to a 50 ml tube.
7. Wash MNC once with PBS with 0.1% BSA by centrifugation at 400 x g for 8 min at 2–8°C.
8. Wash MNC twice with PBS with 0.1% BSA by centrifugation at 225 x g for 8 min at 2–8°C and resuspend the MNC at 2 x 10⁸ MNC per ml in isolation buffer.

For starting samples other than buffy coat please see www.invitrogen.com/cellisolation for recommended MNC preparation procedures.

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

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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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