invitrogen™ | DYNAL® invitrogen bead separations

Cat. no. 114.63D

Rev. no. 000

Dynabeads® FlowComp™ Mouse CD4+CD25+ Treg Cells

– flow compatible and tube-based isolation of mouse regulatory T cells

For research use only.

1. KIT CONTENTS

Dynabeads® FlowComp™ Mouse CD4+CD25+ Treg Cells	
Catalog no.	**114.63D**
Number of cells processed:	**1 x 10^9**
Antibody Mix for Mouse CD4 Cells	2 ml
Mouse Depletion Dynabeads®	2 x 10 ml
FlowComp™ Mouse CD25 Antibody	0.3 ml
FlowComp™ Dynabeads® (mTreg)	1 ml
FlowComp™ Release Buffer	6 ml

For more detailed information of the kit ingredients, please see 'Description of Materials' in section 4.

2. PRODUCT DESCRIPTION

This product is intended for magnetic isolation of $CD4^+CD25^+$ regulatory T cells from mouse secondary lymphoid organs such as spleen and lymph nodes. The supplied protocol describes magnetic labeling and isolation from 1 x 10^8 cells. In the first step untouched $CD4^+$ cells are isolated by depletion of the non-$CD4^+$ cells; Non-$CD4^+$ cells are labeled with Antibody Mix for Mouse CD4 Cells and subsequently depleted with the Mouse Depletion Dynabeads®. In the second step the $CD4^+CD25^+$ regulatory T cells are positively isolated and the beads are removed; $CD4^+CD25^+$ cells are labeled with FlowComp™ Mouse CD25 Antibody and subsequently positively isolated with Dynabeads® FlowComp™ Mouse CD25. This is followed by a simple release step with FlowComp™ Release Buffer to completely remove the beads from the regulatory T cells.

Downstream Applications

Isolated cells may be used directly in any downstream application including flow cytometry, inhibitory assays, cell expansion protocols and in vivo transfer protocols.

Additional Materials Required

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

- Isolation buffer: Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) (e.g. Gibco cat. no. 14190-094) supplemented with 0.1% (wt/vol) BSA and 2mM EDTA (see Technical Recommendations for further information).
- Heat inactivated Fetal Bovine Serum (FBS).
- Media: RPMI or equivalent supplemented with 5% (vol/vol) FBS .
- Mixer allowing both tilting and rotation.
- Magnet (DynaMag™ or Dynal MPC™): See www.invitrogen.com/magnets for magnet recommendations.
- Flow cytometry antibody reagents (optional): For staining of CD25, Rat Anti-Mouse, (Alexa Fluor® 488, Invitrogen Cat. no. RM6020) is recommended (other antibody clones might interfere with the antibody left on the cells). For staining of CD4, we recommend to use CD4, Rat Anti-Mouse, (R-PE, Invitrogen Cat. no. MCD0404).

▲ Critical notes

- Wash the Mouse Depletion Dynabeads® and FlowComp™ Dynabeads® (mTreg) before use.
- 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. For CD25, it is recommended to use CD25, Rat Anti-Mouse, Alexa Fluor® 488 (Invitrogen Cat. no. RM6020) as primary fluorescent antibody. For CD4, it is recommend to use CD4, Rat Anti-Mouse, R-PE (Invitrogen Cat. no. MCD0404). Avoid using secondary antibodies specific for rat antibodies for flow cytometry staining.

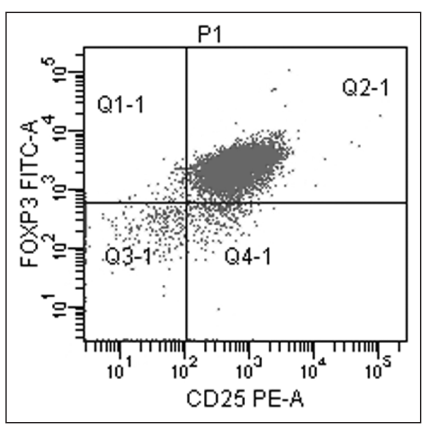

Fig. 1: FOXP3 expression in isolated $CD4^+$ $CD25^+$ Treg cells.

3. PROTOCOL

Approximately 4-10 % 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 labeling and isolation of $CD4^+CD25^+$ regulatory T cells from 1 x 10^8 splenocytes using Dynabeads® FlowComp™ Mouse $CD4^+CD25^+$ Treg Kit.

Dynabeads® Washing Procedure

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 2 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 single cell suspension from lymphoid organs (e.g lymph nodes or spleen).
- Prepare approximately 25 ml isolation buffer pe 1 x 10^8 cells.

Isolation procedure

When working with fewer cells than 1 x 10^8, use the same volumes as indicated. When working with higher cell numbers, scale up **all** reagent and total volumes accordingly (steps 1-24).

Negative isolation of $CD4^+$ cells

1. Resuspend 1 x 10^8 cells in 1 ml isolation buffer and add 200 µl FBS and 200 µl Antibody Mix for Mouse CD4. Mix well and incubate for 20 min at 2 – 8°C (leave tube still).
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 1 ml cold isolation buffer to the cell pellet and resuspend.
5. Add 2 ml pre-washed and resuspended Mouse Depletion Dynabeads® and mix well. Incubate for 15 min at 18-25°C (RT) under rolling and tilting.
6. Resuspend the bead-bound cells by gently pipetting 5 times using a pipette with a narrow tip opening (e.g. a 1,000 µl pipette tip or a 5 ml serological pipette).
7. Add 3 ml isolation buffer and resuspend.
8. Place the tube in the magnet for 2 min.
9. Transfer the supernatant containing the bead-free $CD4^+$ T cells to a new tube.
10. Spin down the cells for 8 min at 350xg and resuspend the cells in 250 µl isolation buffer.

Positive isolation of $CD4^+CD25^+$ cells

11. Per 250 µl (from step 10), add 25 µl FlowComp™ Mouse CD25 Antibody. Mix well and incubate for 20 min at RT.
12. Add 2 ml cold isolation buffer to wash cells, followed by centrifugation for 8 min at 350xg.
13. Remove and discard supernatant and add 250 µl cold isolation buffer to the cell pellet and resuspend.
14. Add 75 µl pre-washed and resuspended FlowComp™ Dynabeads® (mTreg cells) and mix well.
14. Incubate for 15 min at RT with rolling and tilting.
15. Place the tube in the magnet for minimum 2 min. Carefully remove the supernatant containing the $CD4^+CD25^-$ (effector) cells.
16. Remove the tube from the magnet and resuspend the bead-bound cells in 2 ml isolation buffer by pipetting 4-5 times.
17. Place the tube in the magnet for a minimum of 2 min. Remove and discard the supernatant.
18. Remove the tube from the magnet and carefully resuspend the bead-bound cells in 0.5 ml FlowComp™ Release Buffer.
19. Incubate for 20 min at RT with rolling and tilting.
20. Mix the cells by gentle pipetting 10 times and place the tube in the magnet for 2 min.
21. Transfer the supernatant containing the bead-free $CD4^+CD25^+$ cells to a new tube.
22. Put this tube one more time in the magnet and transfer the supernatant to a second new tube.
23. Add 2 ml isolation buffer followed by centrifugation for 8 min at 350xg.
24. Discard the supernatant and resuspend the cell pellet containing the isolated mouse $CD4^+CD25^+$ regulatory T cells in a preferred cell culture medium.

Keep the cells on 2-8°C until further use in downstream applications.

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

Mouse Depletion Dynabeads® are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a polyclonal sheep anti-rat IgG antibody. Supplied at 4 x 10^8 beads per ml in PBS, pH 7.4, containing 0.1% BSA and 0.02% sodium azide.

Dynabeads® FlowComp™ (mTreg cells) are uniform, superparamagnetic polystyrene beads (1 µm diameter). Supplied at approx. 1 x 10^9 beads per ml in PBS, pH 7.4, containing 0.1% BSA and 0.02% sodium azide.

Antibody Mix for Mouse CD4 contains rat IgG antibodies against mouse CD45R (B220), CD11b (Mac-1), Ter-119, CD16/32 and CD8. Supplied in PBS with 0.02% sodium azide.

FlowComp™Mouse CD25 Antibody contains modified monoclonal rat anti-mouse CD25 antibody. Supplied in PBS with 0.5% BSA and 0.02% sodium azide.

FlowComp™ Release Buffer contains release agent supplied in PBS with 0.1% BSA and 2 mM EDTA.

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 RECOMMENDATIONS

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.

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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