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# Dynabeads® FlowComp™ Mouse CD4+CD25+Treg Cells

**Catalog no. 11463D** **Store at 2˚C to 8˚C**

**Rev. Date:** December 2011 (Rev. 002)

## Kit Contents

| Kit contents | Volume |
|---|---|
| Antibody Mix for Mouse CD4 Cells | 2 mL |
| Mouse Depletion Dynabeads® | 2 × 10 mL |
| FlowComp™ Mouse CD25 antibody | 0.3 mL |
| FlowComp™ Dynabeads® (mTreg) | 1 mL |
| FlowComp™ Release Buffer | 6 mL |

**Kit capacity**
~1 × $10^9$ cells

For details on product content, see "Description of Materials" section.

## 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 isolated cells are highly pure, viable, and bead-free. In the first step, the non-CD4+ cells are labeled with Antibody Mix for Mouse CD4 Cells. In the second step Mouse Depletion Dynabeads® are added to remove the non-CD4+ cells. In the third step, FlowComp™ Mouse CD25 antibody and FlowComp™ Dynabeads® are added to the CD4+ T cells to capture the CD4+CD25+ T cells, and in the last step the FlowComp™ Release Buffer is added to remove the beads.

### Downstream Applications

Isolated cells may be used directly in any downstream application including flow cytometry, inhibitory assays, cell expansion protocols using Dynabeads® Mouse T-Activator CD3/CD28 or Dynabeads® Mouse T-Activator CD3/CD28/CD137, and *in vivo* transfer protocols.

## Required Materials

- Magnet (DynaMag™) See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Heat inactivated Fetal Bovine Serum (FBS).
- Media: RPMI or equivalent supplemented with 5% (vol/vol) FBS.
- Isolation Buffer: $Ca^{2+}$ and $Mg^{2+}$ free phosphate buffered saline (PBS) supplemented with 0.1% bovine serum albumin (BSA) and 2 mM EDTA. Alternatively, PBS with 2% fetal calf serum (FCS) and 1 mM EDTA may be used.
- *Optional:* Use primary fluorescent conjugated antibodies for flow cytometry. For staining of CD25, Rat Anti-Mouse, Alexa Fluor® 488 is recommended. For staining of CD4, we recommend to use CD4, Rat Anti-Mouse R-Phycoerythrin (R-PE).

## General Guidelines

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- This product should not be used with magnet MPC™-1.
- Follow the recommended volumes and incubation times.
- Avoid air bubbles (foaming) during pipetting.
- To avoid unspecific labeling of cells during flow staining we recommend using gamma-globulin or Fc blocking reagents prior to staining with primary fluorescent antibody.
- Avoid using secondary antibodies specific for rat antibodies for flow cytometry staining.

## 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 isolation of CD4+CD25+ regulatory T cells from 1 × $10^8$ splenocytes using Dynabeads® FlowComp™ Mouse CD4+CD25+ Treg Kit.

### Prepare Cells

- Prepare a single cell suspension from lymphoid organs (e.g. lymph nodes or spleen) according to "General Guidelines".
- Resuspend the cells at 1 × $10^8$ cells/mL in Isolation Buffer.
- Prepare approximately 25 mL of Isolation Buffer per 1 × $10^8$ cells.

### Wash Dynabeads®

See Table 1 (step 5 and 13) for volume recommendations.

1. Resuspend the Dynabeads® in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads® to a tube.
3. Add the same volume of Isolation Buffer, or at least 1 mL, and resuspend.
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).

### Isolate Untouched Mouse CD4+ T Cells

This protocol is based on 1 × $10^8$ starting cells, but is directly scalable from 5 × $10^7$ to 1 × $10^9$ cells, according to Table 1.

1. To 1 mL prepared sample (1 × $10^8$ cells) add 200 µL FBS and 200 µL antibody Mix for Mouse CD4. Mix well and incubate for 20 min at 2°C to 8°C.
2. Add 4 mL cold Isolation Buffer to wash cells, followed by centrifugation for 8 min at 350 × g.
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°C to 25°C (room temperature) under rolling and tilting.
6. Add 3 mL Isolation Buffer and resuspend the bead-bound cells by gently pipetting 5 times using a pipette with a narrow tip opening.
7. Place the tube in the magnet for 2 min.
8. Transfer the supernatant containing the bead-free CD4+ T cells to a new tube.
9. Spin down the cells for 8 min at 350 × g and resuspend the cells in 250 µL Isolation Buffer.

### Isolate Mouse CD4+CD25+ Cells

10. Add 25 µL FlowComp™ Mouse CD25 antibody per 250 µL cells (from step 9) . Mix well and incubate for 20 min at room temperature.
11. Add 2 mL Isolation Buffer to wash cells, followed by centrifugation for 8 min at 350 × g.
12. Remove and discard supernatant, and add 250 µL cold Isolation Buffer to the cell pellet and resuspend.
13. Add 75 µL pre-washed and resuspended FlowComp™ Dynabeads® (mTreg cells) and mix well.
14. Incubate for 15 min at room temperature 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. *Optional:* Repeat step 16–17 at least once to increase the purity of the isolated cells.

### Release of $CD4^+CD25^+$ regulatory T cells

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 room temperature 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 the tube in the magnet again and transfer the supernatant to a second new tube to remove any residual beads.
23. Add 2 mL Isolation Buffer followed by centrifugation for 8 min at 350 × g.
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°C to 8°C until further use in downstream applications.

Table 1: Examples of volumes for isolation of mouse $CD4^+CD25^+$ T cells.

| Step | Step description | Volumes per $1 \times 10^8$ cells | Volumes per $5 \times 10^8$ cells |
|---|---|---|---|
| | Recommended tube size | 5–7 mL | 50 mL |
| | Recommended magnet | DynaMag™-5 | DynaMag™-50 |
| 1 | Cell volume | 1 mL | 5 mL |
| 1 | FBS | 200 µL | 1 mL |
| 1 | Antibody Mix | 200 µL | 1 mL |
| 2* | Wash cells (Isolation Buffer) | ~4 mL | ~20 mL |
| 4 | Resuspend cells (Isolation Buffer) | 1 mL | 5 mL |
| 5** | Depletion MyOne™ Dynabeads® | 2 mL | 10 mL |
| 6* | Increase volume* | ~3 mL | ~15 mL |
| 9 | Resuspend cells (Isolation Buffer) | 250 µL | 1.25 mL |
| | Recommended tube size | 5–7 ml | 5–7 ml |
| | Recommended magnet | DynaMag™-5 | DynaMag™-5 |
| 10 | Volume cells | 250 µL | 1.25 mL |
| 10 | FlowComp™ Mouse CD25 antibody | 25 µL | 125 µL |
| 11* | Wash cells (Isolation Buffer) | ~2 mL | ~5 mL |
| 12 | Resuspend cells (Isolation Buffer) | 250 µL | 1.25 mL |
| 13** | FlowComp™ Dynabeads® (mTreg) | 75 µL | 375 µL |
| 16–17* | Wash Dynabeads® (Isolation Buffer) | 2 × ~2 mL | 2 × ~5 mL |
| 18 | FlowComp™ Release Buffer | 0.5 mL | 2.5 mL |
| 23* | Wash cells (Isolation Buffer) | ~ 2 mL | ~ 5 mL |

* Adjust the Isolation Buffer volumes to fit to the tube you are using.

** When incubating, tilt and rotate the vial so the cells and beads are kept in the bottom of the tube. Do not perform end-over-end mixing if the volume is small relative to the tube size.

## Description of Materials

Mouse Depletion Dynabeads® contains ~$4 \times 10^8$ beads/mL uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a polyclonal ant-rat antibody. Dynabeads® FlowComp™ (mTreg cells) contains ~$1 \times 10^9$ beads/ mL uniform, superparamagnetic polystyrene beads (1 µm diameter) coated with modified streptavidin. All beads are suspended in PBS, pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. 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 DSB-X biotinylated monoclonal rat anti-mouse CD25 antibody supplied in PBS with 0.5% BSA and 0.02% sodium azide. FlowComp™ Release Buffer contains a modified biotin that out-competes the modified biotin on the antibody to give the cell release from the beads, in PBS with 0.1% BSA and 2 mM EDTA.

**Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

## Related Products

| Product | Cat. no. |
|---|---|
| DynaMag™-5 | 12303D |
| DynaMag™-15 | 12301D |
| DynaMag™-50 | 12302D |
| HulaMixer® Sample Mixer | 15920D |
| CD25, Rat Anti-Mouse Alexa Fluor® 488 | RM6020 |
| CD4, Rat Anti-Mouse R-PE | MCD0404 |
| Dynabeads® Mouse T-Activator CD3/CD28 | 11452D |
| Dynabeads® Mouse T-Activator CD3/CD28/CD137 | 11454D |

REF on labels is the symbol for catalog number.

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