

Dynabeads® Untouched™ Human CD4 T Cells

Catalog nos. 11346D, 11352D

Store at 2 °C to 8 °C

Rev. Date: July 2012 (Rev. 005)

Kit Contents

Kit contents	Cat. no. 11346D	Cat. no. 11352D
Depletion MyOne™ SA Dynabeads®	2 × 5 mL	2 mL
Antibody Mix (Human CD4 ⁺ T Cells)	2 mL	0.4 mL

Kit capacity

PBMC for 11346D: ~1 × 10⁹

PBMC for 11352D: ~2 × 10⁸

Depletion MyOne™ Dynabeads® contains 20 mg beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. Antibody Mix contains monoclonal anti-human IgG antibodies in PBS with 0.5% BSA and 0.02% sodium azide. **Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

This product is intended for isolation of untouched human CD4⁺ T cells from peripheral blood mononuclear cells (PBMC) by depleting B cells, NK cells, monocytes, platelets, dendritic cells, CD8⁺ T cells, granulocytes and erythrocytes. Isolated CD4⁺ T cells are bead- and antibody-free and are suitable for any downstream application (fig. 1).

A mixture of mouse IgG antibodies against the non-CD4⁺ T cells is added to the starting sample and bind to the cells. Depletion MyOne™ Dynabeads® are added and binds to the antibody labelled cells during a short incubation. The bead-bound cells are subsequently separated on a magnet and discarded. The supernatant contains the untouched human CD4⁺ T cells.

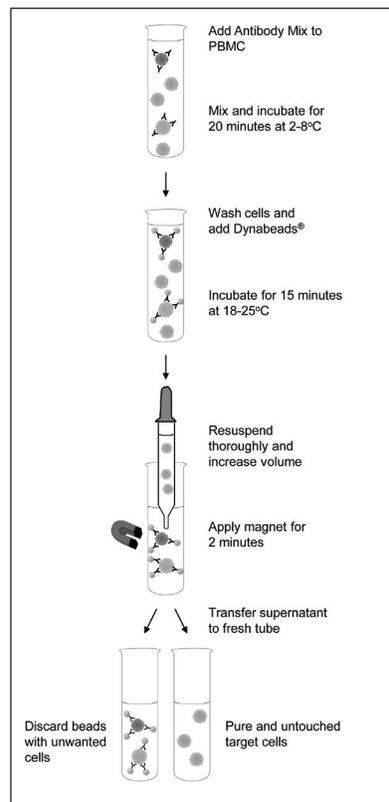


Figure 1: Isolation principle for Untouched CD4⁺ cells

Downstream Applications

Isolated CD4⁺ T cells can be used in any application, e.g.:

- Studies on CD4⁺ T cell proliferation, apoptosis and induction of anergy.
- Studies on antigen specific T cells.
- Studies on regulation of CD4⁺ T cell cytokine expression.
- Flow cytometry/FACS sorting. Isolated cells can be activated/expanded using Dynabeads® Human T-Activator CD3/CD28 (polyclonal activation) or Dynabeads® Human T-Activator CD3/CD28/CD137 (antigen-specific activation).

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixing device with tilting and rotation, e.g. HulaMixer® Sample Mixer.
- Heat inactivated Fetal Bovine Serum (FBS)/Fetal Calf Serum (FCS).
- Isolation Buffer: PBS (Ca²⁺ and Mg²⁺ free) supplemented with 0.1% BSA and 2 mM EDTA.
Note: BSA can be replaced by human serum albumin (HSA) or 2% FBS/FCS. EDTA can be replaced by 0.6% sodium citrate.
- Lymphoprep® for PBMC preparation (Axis Shield PoC, Norway, www.axis-shield-poc.com).

General Guidelines

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads® do not settle in the tube.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Follow the recommended volumes and incubation times.
- Avoid air bubbles (foaming) during pipetting.
- Keep the buffers cold.

Protocol

Wash Dynabeads®

See Table 1 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).

Prepare Cells

Prepare a PBMC suspension according to “General Guidelines”. Resuspend the cells at 1×10^8 cells/mL in Isolation Buffer.

Isolation Procedure

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

1. Transfer 500 μ L (5×10^7) PBMC in Isolation Buffer to a tube.
2. Add 100 μ L heat inactivated FBS/FCS.
3. Add 100 μ L of Antibody Mix.
4. Mix well and incubate for 20 min at 2°C to 8°C.
5. Wash the cells by adding 4 mL Isolation Buffer. Mix well by tilting the tube several times and centrifuge at $350 \times g$ for 8 min at 2°C to 8°C. Discard the supernatant.
6. Resuspend the cells in 500 μ L Isolation Buffer.
7. Add 500 μ L pre-washed Dynabeads®.
8. Incubate for 15 min at 18°C to 25°C with gentle tilting and rotation.
9. Add 4 mL Isolation Buffer. (When working with lower cell volumes, never use less than 1 mL Isolation Buffer).
10. Resuspend the bead-bound cells thoroughly by pipetting >10 times using a pipette with a narrow tip opening. Avoid foaming.
11. Place the tube in the magnet for 2 min. Transfer the supernatant containing the untouched human CD4⁺ T cells, to a new larger tube.
12. Add 4 mL Isolation Buffer to the tube containing the Dynabeads® and resuspend the bead-bound cells by pipetting as described in step 10.
13. Place the tube in the magnet for 2 min.
14. Combine the two supernatants.
15. *Optional:* To remove residual beads; place the tube in the magnet for 2 min and transfer cells to a new tube.

Table 1: Volumes for isolation of human CD4⁺ T cells. This protocol is scalable from 1×10^7 to 5×10^8 PBMC.

Step	Step description	Volumes per 5×10^7 PBMC	Volumes per 2×10^8 PBMC
	Recommended tube	5–7 mL tubes	15 mL tubes
	Recommended magnet	DynaMag™-5	DynaMag™-15
1	Cell volume	500 μ L	2 mL
2	FBS/FCS	100 μ L	400 μ L
3	Antibody Mix	100 μ L	400 μ L
5*	Wash cells (Isolation Buffer)	~4 mL	~10 mL
6	Resuspend cells (Isolation Buffer)	500 μ L	2 mL
7**	Depletion Dynabeads®	500 μ L	2 mL
9–12*	Increase volume (Isolation Buffer)	$2 \times \sim 4$ mL	$2 \times \sim 10$ mL

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

** When incubating, tilt and rotate 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

Depletion MyOne™ Dynabeads® (20 mg/mL) are uniform, super-paramagnetic polymer beads (1.0 μ m diameter) coated with a monoclonal human IgG4 anti-mouse IgG antibody, which recognizes all mouse IgG subclasses and is Fc-specific. The Antibody Mix contains mouse IgG antibodies towards human CD8, CD14, CD16 (specific for CD16a and CD16b), CD19, CD36, CD56, CDw123 and CD235a (Glycophorin A).

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
Dynabeads® Human T-Activator CD3/CD28	11131D
Dynabeads® Human T-Activator CD3/CD2/CD137	11162D
HulaMixer® Sample Mixer	15920D
Phosphate Buffered Saline	10010-023

[REF] on labels is the symbol for catalog number.

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