

Dynabeads[®] Untouched[™] Human T Cells

Catalog no. 11344D

Store at 2 °C to 8 °C

Rev. Date: February 2012 (Rev. 002)

Kit Contents

Kit contents	Volume
Depletion Dynabeads [®]	2 × 5 mL
Antibody Mix (Human T Cells)	2 mL

Kit capacity

PBMC: ~1 × 10⁹

Depletion Dynabeads[®] contains 4 × 10⁸ 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 mouse 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 T cells from peripheral blood mononuclear cells (PBMC) by depleting B cells, NK cells, monocytes, platelets, dendritic cells, granulocytes and erythrocytes. Isolated 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-T cells is added to the starting sample. Depletion Dynabeads[®] are added and bind to the antibody-labeled cells during a short incubation. The bead-bound cells are subsequently separated on a magnet and discarded. The supernatant contains the untouched human T cells.

Downstream Applications

Isolated T cells can be used in any downstream application e.g. flow cytometry, T cell culture, T cell activation and expansion, functional assays, molecular studies.

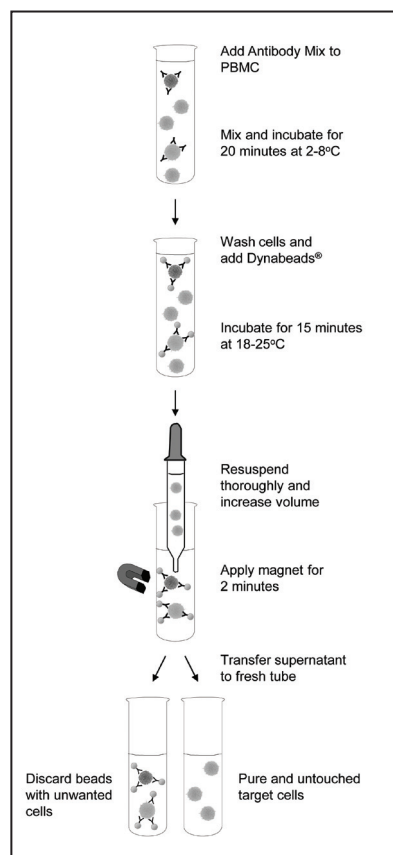


Figure 1: Isolation principle for untouched T Cells.

Required Materials

- Magnet (DynaMag[™]) 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.

- Resuspend the Dynabeads[®] in the vial (i.e vortex for >30 sec, or tilt and rotate for 5 min).
- Transfer the desired volume of Dynabeads[®] to a tube.
- Add the same volume of Isolation Buffer, or at least 1 mL, and resuspend.
- Place the tube in a magnet for 1 min and discard the supernatant.
- 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 × 10⁸ 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 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.

Table 1: Volumes for isolation of human 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 Dynabeads® are uniform, superparamagnetic polymer beads (4,5 μ m 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 Mix contains mouse IgG antibodies for CD14, CD16 (specific for CD16a and CD16b), CD19, CD36, CD56, CDw123 and CD235a (Glycophorin A). Supplied in PBS with 0.5% BSA and 0.02% sodium azide.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Phosphate Buffered Saline	10010-023

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

Limited Use Label License

The purchase of this product conveys to the purchaser the limited, nontransferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Manufactured by Life Technologies AS, Norway. Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

SPEC-06428

©2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners, except where otherwise stated. Life Technologies and/or its affiliate(s) disclaim all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall Life Technologies and/or its affiliate(s) be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

 life technologies™