

Dynabeads® Human DC Enrichment Kit

Catalog no. 11308D

Store at 2 to 8°C

Rev. Date: November 2011 (Rev. 002)

Kit Contents

Kit contents	Volume
Depletion MyOne™ SA Dynabeads®	2 × 10 mL
Antibody Mix (for DC Kit)	4 mL

Kit capacity

MNC: ~2 × 10⁹

Depletion MyOne™ Dynabeads® contains 10 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 biotinylated monoclonal anti-human antibodies in PBS with 0.5% BSA and 0.02% sodium azide.

Product Description

This product is intended for enrichment of untouched Dendritic Cells (DCs) by depleting T cells, B cells, monocytes/macrophages, NK cells, erythrocytes and most granulocytes from blood mononuclear cells (MNC). The DC enriched population is bead- and antibody-free and intended for further isolation of any DC subpopulation by flow sorting. This kit provides high recovery of lineage specific markers (Lin-) CD4⁺ cells and is therefore suitable for further isolation of any DC subpopulation, e.g. myeloid and plasmacytoid DCs (fig. 1).

Add a mixture of biotinylated monoclonal antibodies against non-DC cells to the MNC.

Add Depletion MyOne™ SA Dynabeads® and allow them to bind to the non-DCs during a short incubation. Separate the bead-bound cells with a magnet. Discard the bead-bound cells and use the remaining untouched, enriched cell population for any application.

Downstream Applications

The DC enriched cell population can be used for further isolation of DC subpopulations to high purity using flow sorting.

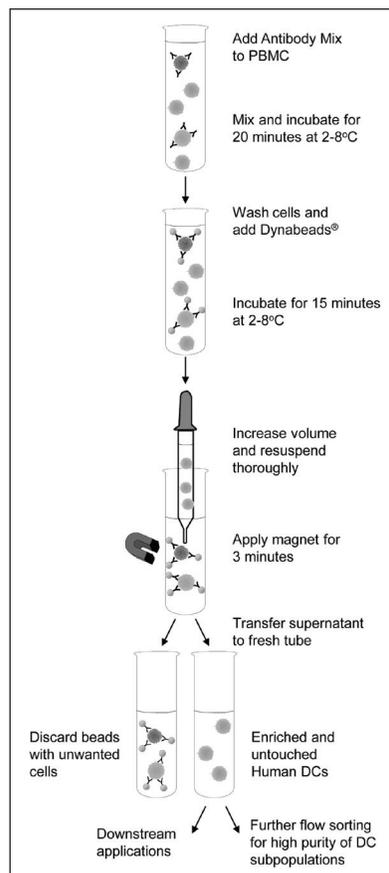


Figure 1: Enrichment principle for untouched DCs.

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 MNC 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 MPC™-1 (cat.no. 12001D).
- Follow the recommended volumes and incubation times.
- Avoid air bubbles (foaming) during pipetting.
- It is important to keep cells and buffers cold when working with DCs.
- Do not use buffers or additives (i.e. FCS) containing biotin since this may reduce efficiency of depletion.

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 mix.
- Place the tube in a magnet for 3 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 MNC suspension according to “General Guidelines”
- Resuspend the cells at 1 × 10⁸ cells/mL in Buffer.

Enrichment of DCs

This protocol is based on enrichment from 5×10^7 MNC, but is scalable from 1×10^7 to 2×10^9 cells, according to Table 1. When working with other cell numbers, scale up all reagents and volumes accordingly. Keep the temperature low to reduce DC depletion. Pre-cool the buffer to 2°C to 8°C.

1. Transfer 500 μ L (5×10^7) MNC in Isolation Buffer to a tube.
2. Add 100 μ L of Antibody Mix.
3. Mix well and incubate for 20 min at 2°C to 8°C.
4. Wash the cells by adding 10 mL Isolation Buffer. Mix well by tilting the tube several times and centrifuge at $300 \times g$ for 10 min at 2°C to 8°C. Discard the supernatant.
5. Resuspend the cells in 4.5 mL Isolation Buffer.
6. Add 500 μ L pre-washed and resuspended Dynabeads®.
7. Incubate for 15 min at 2°C to 8°C with gentle tilting and rotation.
8. Add 5 mL Isolation Buffer.
9. Resuspend the bead-bound cells by thoroughly pipetting >10 times using a pipette with a narrow tip opening. Avoid foaming.
10. Place the tube in the magnet for 3 min.
11. Transfer the supernatant to a new larger tube.
12. Add 5 mL Isolation Buffer to the tube containing the Dynabeads® and resuspend the bead-bound cells by pipetting as described in step 9.
13. Place the tube in the magnet for 3 min.
14. Combine the two supernatants.
15. Optional: To remove residual beads; place the tube in the magnet for 3 min and transfer cells to a new tube.

The supernatant contains the DC enriched cell population.

Table 1: Volume requirements for DC enrichment.

Step	Step description	Volumes per 5×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube	15 mL tubes	50 mL tubes
	Recommended magnet	DynaMag™-15	DynaMag™-50
1	Cell volume	500 μ L	2 mL
2	Antibody Mix	100 μ L	400 μ L
4*	Wash cells (Isolation Buffer)	~10 mL	~35 mL
5	Resuspend cells (Isolation Buffer)	4.5 mL	18 mL
6**	Depletion Dynabeads®	500 μ L	2 mL
8*	Increase volume (Isolation Buffer)	~5 mL	~20 mL
12*	Increase volume (Isolation Buffer)	~5 mL	~20 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

Depletion MyOne™ SA Dynabeads® are uniform, superparamagnetic polystyrene beads (1.0 μ m diameter) coated with streptavidin (SA). The Antibody Mix contains an optimized mixture of biotinylated monoclonal antibodies against CD3, CD14, CD16, CD19, CD56 and Glycophorin A.

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.

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