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Dynabeads® CD34 Positive Isolation Kit

Catalog no. 11301D **Store at 2 to 8°C**

Rev. Date: November 2011 (Rev. 004)

Kit Contents

Kit contents	Volume
Dynabeads® CD34	5 mL
DETACHaBEAD® CD34	5 mL

Kit capacity
MNC: ~5 × 10^9

Dynabeads® CD34 contains 4 × 10^8 beads/mL in phosphate buffered saline (PBS), with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative. DETACHaBEAD® CD34 contains a polyclonal anti-Fab antibody in 0.15 M PBS.

Product Description

This product is intended for positive magnetic isolation of $CD34^+$ cells from mononuclear cells (MNCs) from human bone marrow, peripheral blood or cord blood. In the first step $CD34^+$ cells are captured by the Dynabeads® and isolated using a magnet. In the second step beads are removed from the cells.

Isolated cells are bead- and antibody-free, phenotypically unaltered, and suitable for any downstream application including flow cytometry, functional studies and cell culture.

Required Materials

- Magnet (DynaMag™): See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Samp4 x le Mixer).
- Isolation Buffer: PBS (Ca^{2+} and Mg^{2+} free with 0.1% BSA 2 mM EDTA, pH 7.4. **Note:** BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by 0.6% sodium citrate. PBS containing Ca^{2+} or Mg^{2+} is not recommended, except when required for DNase treatment.
- Culture Medium: e.g. RPMI 1640 with 2% FCS.
- DNase I (when required).

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).
- Avoid air bubbles (foaming) during pipetting.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.

Protocol

Wash Dynabeads®

See Table 1 for volume recommendations.

1. Resuspend the Dynabeads® in the vial (vortex >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 mix.
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 $CD34^+$ Stem Cells

The isolation and release protocol is based on 1 mL MNC (4 × 10^7 – 1 × 10^8 cells/mL) as starting sample, but can be scaled up according to Table 1.

1. Resuspend the prepared cell sample thoroughly with a narrow pipette.
2. Add 100 µL washed and resuspended Dynabeads® to the prepared sample and vortex 2–3 sec.
3. Incubate at 2°C to 8°C for 30 min with gentle tilting and rotation.
4. Fill the tube with cold buffer to the height of the magnet (or at least 1 mL) and resuspend the cell-bead complexes.
5. Place the tube in a magnet for 2 min and discard the supernatant.
6. Resuspend the bead-bound cells in 2 mL buffer by vortexing or pipetting. Separate on a magnet for 1 min.
7. Repeat step 6 twice to increase the purity.
8. Resuspend the bead-bound cells in 100 µL buffer.

Prepare MNC

- Prepare MNC according to "General Guidelines".
- Prepare MNC to 4 × 10^7 – 1 × 10^8 MNC/mL. Allways use fresh samples.

Release $CD34^+$ Stem Cells

Tubes and buffers can be kept at room temperature for the release procedure.

9. Add 100 µL DETACHaBEAD® (never use less than 100 µL DETACHaBEAD®).
10. Incubate at room temperature for 45 min with tilting and rotation. Keep the sample in the bottom of the tube.
11. Add 2 mL Isolation Buffer and vortex 2–3 sec to enhance detachment of beads from the cells.
12. Place the tube in a magnet for 2 min.
13. Transfer the supernatant containing released cells to a fresh tube. To obtain residual cells, wash the beads 3 times in 500 µL Isolation Buffer and pool the supernatants.
14. Wash the cells thoroughly by resuspending in 10 mL Isolation Buffer. Centrifuge for 10 min at 400 × g to remove excess DETACHaBEAD®.
15. Resuspend the cells in Isolation Buffer or Culture Medium.

The isolated cells are pure, viable, and free from antibody bound to the surface, and may be used in any downstream application.

Table 1: Volumes for human CD34+ stem cell isolation from MNC. Use 4×10^7 – 1×10^8 cells/mL. For lower volumes than 1 mL, use the same volumes as the 1 mL column. For higher cell numbers, scale up the volumes accordingly.

Step	Step description	Cell volume: 1 mL	Cell volume: 5 mL
	Recommended tube size	5 mL tube	15 mL tube
	Recommended magnet	DynaMag™-5	DynaMag™-15
2	Dynabeads® CD34	100 µL	500 µL
4	Resuspend cells (Isolation Buffer)	~3 mL	~8 mL
6–7	Wash cells (Isolation Buffer)	2 mL × 2	10 mL × 2
8	Resuspend cells (Isolation Buffer)	100 µL	500 µL
9	Release cells (DETACHaBEAD®)	100 µL	500 µL
11	Increase cell volume (Isolation Buffer)	2 mL	10 mL
13	Collect residual cells	500 µL × 3	2.5 mL × 3
14*	Remove DETACHaBEAD®	Increase to 10 mL	Increase to 30 mL

*Transfer the sample to a larger tube that is more appropriate to the volume (e.g. 15 mL tube and 50 mL tube, respectively).

Description of Materials

Dynabeads® CD34 are uniform, superparamagnetic polymer beads (4.5 µm diameter) coated with a primary monoclonal antibody specific for the CD34 membrane antigen predominantly expressed on human hematopoietic progenitor cells and endothelialprogenitor cells. DETACHaBEAD® CD34 is a polyclonal anti-Fab antibody specific for the CD34 antibody on the Dynabeads® CD34.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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

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