invitrogen
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Dynabeads® Untouched™ Human Monocytes

Catalog no. 11350D **Store at 2˚C to 8˚C**

Rev. Date: February 2012 (Rev. 001)

Kit Contents

Kit contents	Volume
Depletion MyOne™ SA Dynabeads®	2 × 5 mL
Antibody Mix (Human Monocytes)	2 mL

Kit capacity
PBMC: ~1 × 10^9

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. The Blocking Reagent contains aggregated gamma globulin in 0.9% NaCl.
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 monocytes by depletion of non-monocytes (T cells, B cells, NK cells, dendritic cells, erythrocytes, granulocytes and macrophages) from peripheral blood mononuclear cells (PBMC). Isolated monocytes are bead- and antibody-free and are suitable for any downstream application (fig. 1).

Add blocking reagent and a mixture of biotinylated monoclonal antibodies (Antibody Mix) against the non-monocytes to the starting sample. Add Depletion MyOne™ SA Dynabeads® to bind the non monocytes during a short incubation.

Separate the bead-bound cells with a magnet. Discard the bead-bound cells and use the remaining, untouched human monocytes for any application.

Downstream Applications

Isolated human monocytes can be used in any application, e.g.: Cell culture, generation of monocyte-derived dendritic cells (Mo-DC), functional assays, molecular studies and flow cytometry.

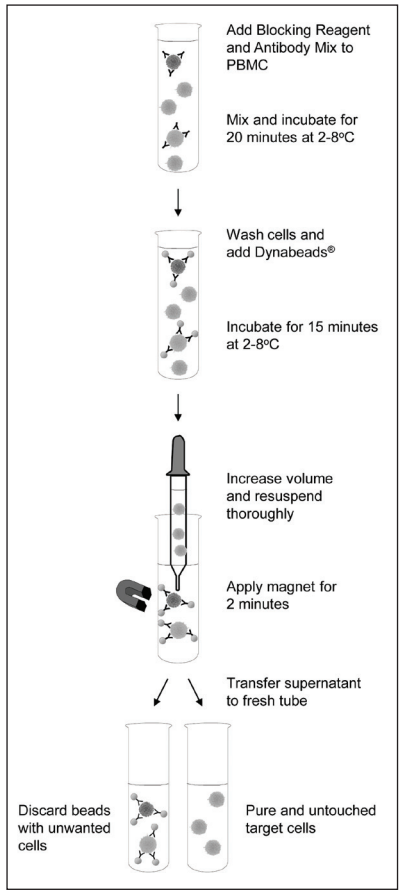

Figure 1: Isolation principle for untouched Monocytes.

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 (Ca^{2+} and Mg^{2+} 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.
- It is important to keep cells and buffers cold when working with monocytes.

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 µL (5×10^7) PBMC in Isolation Buffer to a tube.
2. Add 100 µL Blocking Reagent.
3. Add 100 µL 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 × g for 8 min at 2°C to 8°C. Discard the supernatant.
6. Resuspend the cells in 500 µL Isolation Buffer.
7. Add 500 µL pre-washed Dynabeads®.
8. Incubate for 15 min at 2°C to 8°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 monocytes, 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 monocytes. 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 µL	2 mL
2	Blocking reagent	100 µL	400 µL
3	Antibody Mix	100 µL	400 µL
5*	Wash cells (Isolation Buffer)	~4 mL	~10 mL
6	Resuspend cells (Isolation Buffer)	500 µL	2 mL
7**	Depletion Dynabeads®	500 µL	2 mL
9–12*	Increase volume (Isolation Buffer)	2 × ~4 mL	2 × ~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® are uniform, superparamagnetic polystyrene beads (1.0 µm diameter) coated with streptavidin (SA). The Antibody Mix contains biotinylated mouse IgG antibodies for CD3, CD7, CD16 (specific for CD16a and CD16b), CD19, CD56, CDw123 and CD235a (Glycophorin A). The Blocking Reagent is aggregated gamma globulin in 0.9% NaCl. The gamma globulin may precipitate in solution due to high concentration. This is not contamination and the solution can be used after mixing. The Blocking Reagent is added to block the FC- receptors on the monocytes.

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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