Dynabeads™ Untouched™ Human Monocytes Kit

Catalog Number 11350D

Pub. No. MAN0025622 Rev. A.0

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. 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. The kit includes:

- Depletion MyOne™ SA Dynabeads™
- Antibody Mix (Human Monocytes)
- Blocking Reagent (Human Monocytes)

Depletion MyOne™ SA Dynabeads™ are uniform, superparamagnetic polystyrene beads (1.0 µm diameter) coated with streptavidin (SA). Depletion MyOne™ SA Dynabeads™ contain 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 mouse IgG antibodies for CD3, CD7, CD16 (specific for CD16a and CD16b), CD19, CD56, CDw123, and CD235a (Glycophorin A). Antibody Mix contains biotinylated monoclonal anti-human antibodies in PBS with 0.5% BSA and 0.02% sodium azide.

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.

Contents and storage

Table 1 Contents and storage

Contents	Amount	Storage
Depletion MyOne™ SA Dynabeads™[1]	2 x 5 mL	2°C to 8°C
Antibody Mix (Human Monocytes)[1]	2 mL	
Blocking Reagent (Human Monocytes)	2 mL	

[1] Contains sodium azide.

CAUTION! Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Required materials not supplied

- DynaMag™ magnet. For recommendations, see http://www.lifetechnologies.com/magnets.
- Mixing device with tilting and rotation, e.g. HulaMixer™ Sample Mixer.
- Lymphoprep™ for PBMC preparation.
- 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% Fetal Bovine Serum (FBS)/Fetal Calf Serum (FCS). EDTA can be replaced by 0.6% sodium citrate.

General guidelines

- Visit http://www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- Use a mixer that provides tilting and rotation of the tubes to ensure that the Dynabeads do not settle in the tube.
- Follow the recommended volumes and incubation times.
- It is important to keep cells and buffers cold when working with monocytes.

Workflow

Isolation of untouched monocytes

Label the cells

- Add the Blocking Reagent and Antibody Mix to PBMC.
- Incubate for 20 minutes.

Wash the cells

- Wash the cells with Isolation Buffer.
- Centrifuge for 8 minutes, then resuspend.

Isolate monocytes

- Add the Dynabeads.
- Incubate for 15 minutes.
- Apply the magnet for 2 minutes.
- Transfer the supernatant with the monocytes to a fresh tube.

Recommended volumes for isolation

Table 2 Volumes for isolation of human monocytes

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 Magnet	DynaMag™-15 Magnet
Cell volume	500 µL	2 mL
Blocking Reagent	100 µL	400 µL
Antibody Mix	100 µL	400 µL
Wash cells (Isolation Buffer)[1]	~4 mL	~10 mL
Resuspend cells (Isolation Buffer)	500 µL	2 mL
Depletion MyOne™ SA Dynabeads™[2]	500 µL	2 mL
Increase volume (Isolation Buffer)[1]	2 × ~4 mL	2 × ~10 mL

[1] Adjust the Isolation Buffer volumes to fit to the tube you are using.
[2] 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.

Methods

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 2.

Wash the Dynabeads

See Table 2 for volume recommendations.

1. Resuspend the Dynabeads in the vial (vortex for >30 seconds, or tilt and rotate for 5 minutes).
2. Transfer 500 µL of Dynabeads to a tube.
3. Add 1 mL of Isolation Buffer and resuspend.
4. Place the tube in a magnet for 1 minute, then discard the supernatant.
5. Remove the tube from the magnet, then resuspend the washed Dynabeads in 500 µL of Isolation Buffer.

Prepare the cells

Prepare a PBMC suspension according to "General guidelines" on page 1. Resuspend the cells at 1×10^8 cells/mL in Isolation Buffer.

Label the cells

1. Transfer 500 µL (5×10^7) PBMC in Isolation Buffer to a tube.
2. Add 100 µL of Blocking Reagent.
3. Add 100 µL of Antibody Mix.
4. Mix well, then incubate for 20 minutes at 2°C to 8°C.

Wash the cells

1. Wash the cells by adding 4 mL of Isolation Buffer. Mix well by tilting the tube several times.
2. Centrifuge at 350 × *g* for 8 minutes at 2°C to 8°C. Discard the supernatant.
3. Resuspend the cells in 500 µL of Isolation Buffer.

Isolate monocytes

1. Add 500 µL of pre-washed Dynabeads.
2. Incubate for 15 minutes at 2°C to 8°C with gentle tilting and rotation.
3. Add 4 mL of Isolation Buffer.

   Note: When working with lower cell volumes, never use less than 1 mL of Isolation Buffer.
4. Resuspend the bead-bound cells thoroughly by pipetting >10 times using a pipette with a narrow tip opening. Avoid foaming.
5. Place the tube in the magnet for 2 minutes. Transfer the supernatant containing the untouched human monocytes, to a new larger tube.

Collect the remaining monocytes (optional)

1. Add 4 mL of Isolation Buffer to the tube containing the Dynabeads, then resuspend the bead-bound cells by pipetting as described in step 4 of the "Isolate monocytes" procedure.
2. Place the tube in the magnet for 2 minutes.
3. Collect the supernatant and combine with the collected monocytes (from step 5 of the "Isolate monocytes" procedure).
4. *Optional:* To remove residual beads, place the tube with the isolated monocytes in the magnet for 2 minutes, then transfer cells to a new tube.

Related products

Table 3 Related products

Item	Source
DynaMag™-5 Magnet	12303D
DynaMag™-15 Magnet	12301D
DynaMag™-50 Magnet	12302D
HulaMixer™ Sample Mixer	15920D
Phosphate Buffered Saline	10010-023

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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania Thermo Fisher Scientific Baltics UAB complies with Quality System Standards ISO 9001 and ISO 13485.

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0025622

Revision	Date	Description
A.0	7 September 2021	• Initial release with new publication number format. Supersedes version dated February 2012 (Rev. 001). • Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty. • Added Blocking Reagent (Human Monocytes) to kit contents. • Removed guidelines regarding Cat. No. 12001D.