Dynabeads™ Untouched™ Human B Cells Kit

Catalog Numbers 11351D

Pub. No. MAN0025887 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Dynabeads™ Untouched™ Human B Cells Kit is intended for isolation of untouched human B cells by depletion of non-B cells (T cells, monocytes, NK cells, macrophages, granulocytes, plasma cells, platelets, and erythrocytes) from peripheral blood mononuclear cells (PBMC). Isolated B cells are bead- and antibody-free, and are suitable for use in any downstream application, including: antigen recognition by B cells and interaction with other cells of the immune system, analysis of B cell immunoglobulin class switching and somatic hypermutation, analysis of B cell activation, proliferation and differentiation; B cell signalling pathway studies and flow cytometry.

The kit includes:

- Depletion MyOne™ SA Dynabeads™ magnetic beads—Uniform, superparamagnetic polystyrene beads (1.0 µm diameter) coated with streptavidin (SA).
- Antibody Mix (Human B Cells)—Contains biotinylated mouse IgG antibodies for CD2, CD14, CD16 (specific for CD16a and CD16b), CD36, CD43 and CD235a (Glycophorin A).

The procedure is performed in three simple steps. Cell samples are incubated with a mixture of biotinylated antibodies directed against non-B cells (Antibody Mix). The cells are subsequently washed, then combined with Depletion MyOne™ SA Dynabeads™ magnetic beads. Following a brief incubation, bead-bound cells (non-B cells) are removed using a magnet, then discarded. The resulting supernatant contains untouched human B cells.

Contents and storage

This kit has capacity for ~1 × 10^9 PBMC.

Contents	Amount	Storage
Depletion MyOne™ SA Dynabeads™ magnetic beads (10 mg beads/mL)[1]	2 × 5 mL	2°C to 8°C
Antibody Mix (Human B Cells)[2]	2 mL	

[1] The beads are supplied in phosphate buffered saline (PBS, pH 7.4) with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative.

[2] Antibody Mix contains biotinylated monoclonal anti-human antibodies in PBS with 0.5% BSA and 0.02% sodium azide.

Required materials not supplied

- DynaMag™ magnet. For recommendations, go to thermofisher.com/magnets.
- Mixing device with tilting and rotation, e.g. HulaMixer™ Sample Mixer (Cat. No. 15920D).
- Lymphoprep™ density gradient medium for PBMC preparation.
- Isolation Buffer: PBS, pH 7.4 (Cat. No. 10010023), 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

- Use a mixer that provides tilting and rotation of the tubes to ensure that the Dynabeads™ magnetic beads do not settle in the tube.
- Follow the recommended volumes and incubation times.
- Avoid air bubbles (foaming) during pipetting.
- Keep the buffers cold.

Guidelines for sample preparation

- Go to thermofisher.com/samplepreparation for recommended sample preparation procedures.
- Resuspend cells at a density 1 × 10^8 cells/mL in Isolation Buffer.

Workflow

Isolation of untouched human B cells

Label the cells

a. Add the Antibody Mix to PBMC.
b. Mix, then incubate for 20 minutes.

Wash the cells

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

Isolate human B cells

a. Add the Dynabeads™ magnetic beads.
b. Incubate for 15 minutes.
c. Apply the magnet for 2 minutes.
d. Transfer the supernatant with the human B cells to a new tube.

Recommended volumes for isolation

This protocol is scalable from 1 × 10^7 to 5 × 10^8 PBMC.

Table 1 Volumes for human B cells

Description	Volume per 5 × 10^7 PBMC	Volume per 5 × 10^8 PBMC
Recommended tube	10–15 mL tubes	50 mL tubes
Recommended magnet	DynaMag™-5 Magnet	DynaMag™–50 Magnet
Cell volume	500 µL	5 mL
Antibody Mix	100 µL	1 mL
Wash cells (Isolation Buffer)[1]	~4 mL	~40 mL
Resuspend cells (Isolation Buffer)	500 µL	5 mL
Depletion MyOne™ SA Dynabeads™ magnetic beads[2]	500 µL	5 mL
Increase volume (Isolation Buffer)[1]	2 × ~4 mL	2 × ~40 mL

[1] Adjust the volume of Isolation Buffer according to the size of 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.

Wash the Dynabeads™ magnetic beads

This protocol is based on 5 × 10^7 PBMC, but is directly scalable from 1 × 10^7 to 5 × 10^8 cells (see Table 1 on page 2).

1. Vortex the Dynabeads™ magnetic beads for at least 30 seconds, or tilt and rotate the vial for 5 minutes to ensure the beads are fully resuspended.
2. Transfer 500 µL of Dynabeads™ magnetic beads to a tube.
3. Add 1 mL of Isolation Buffer, then gently mix.
4. Place the tube on a magnetic rack for 1 minute.
5. Keeping the tube on the magnet, carefully remove then discard the supernatant.
6. Remove the tube from the magnet, then resuspend the washed Dynabeads™ magnetic beads in 500 µL of Isolation Buffer.

Label the cells

Prepare cell samples before starting this procedure. See "Guidelines for sample preparation" on page 1.

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

Wash the cells

1. Add 4 mL of Isolation Buffer to the labeled cells, then mix thoroughly 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 human B cells

1. Add 500 µL of pre-washed Dynabeads™ magnetic beads to the cell suspension.
2. Incubate for 15 minutes at 18°C to 25°C with gentle tilting and rotation.
3. Add 4 mL of Isolation Buffer.

   Note: When working with lower cell volumes, do not use less than 1 mL of Isolation Buffer.
4. Using a pipette with a narrow tip opening, thoroughly resuspend bead-bound cells by pipetting up and down at least 10 times. Avoid foaming.
5. Place the tube on the magnet for 2 minutes.
6. Keeping the tube on the magnet, transfer the supernatant, containing the untouched human B cells, to a new tube.

   Do not discard the tube containing the Dynabeads™ magnetic beads.
7. *(Optional)* To remove residual beads, place the tube with the isolated human B cells on the magnet for 2 minutes, then transfer the cells to a new tube.

(Optional) Collect the remaining human B cells

Collecting the remaining untouched B cells a second time can increase the yield, but might lower the purity.

1. Add 4 mL of Isolation Buffer to the tube containing the Dynabeads™ magnetic beads.
2. Using a pipette with a narrow tip opening, thoroughly resuspend bead-bound cells by pipetting up and down at least 10 times. Avoid foaming.
3. Place the tube on the magnet for 2 minutes.
4. Keeping the tube on the magnet, collect the supernatant, then combine with the previously collected human B cells.

Related products

Product	Cat. No.
DynaMag™-5 Magnet	12303D
DynaMag™-15 Magnet	12301D
DynaMag™–50 Magnet	12302D
HulaMixer™ Sample Mixer	15920D
PBS, pH 7.4	10010023

Limited product warranty

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
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.

Revision history: Pub. No. MAN0025887

Revision	Date	Description
A.0	16 November 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. • Volumes were specified in wash steps (see “Wash the Dynabeads™ magnetic beads” on page 3). • Regulatory statement was updated to "For Research Use Only. Not for use in diagnostic procedures." • Two products were deleted from “Related products” on page 3.