

# Dynabeads® Untouched™ Human B Cells

Catalog no. 11351D

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 B Cells)	2 mL

### Kit capacity

PBMC:  $\sim 1 \times 10^9$

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.

**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 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 (PBMCs). Isolated B cells are bead- and antibodyfree and are suitable for any downstream application (fig. 1).

A mixture of biotinylated antibodies against the non-B cells is added to the starting sample and allowed to bind to the cells. Depletion MyOne™ SA Dynabeads® are added and bind to the antibody labelled cells during a short incubation. The bead-bound cells are subsequently separated

on a magnet and discarded. The supernatant contains the untouched human B cells.

## Downstream Applications

Isolated Human B cells can be used in any application, e.g.: Antigen presentation 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.

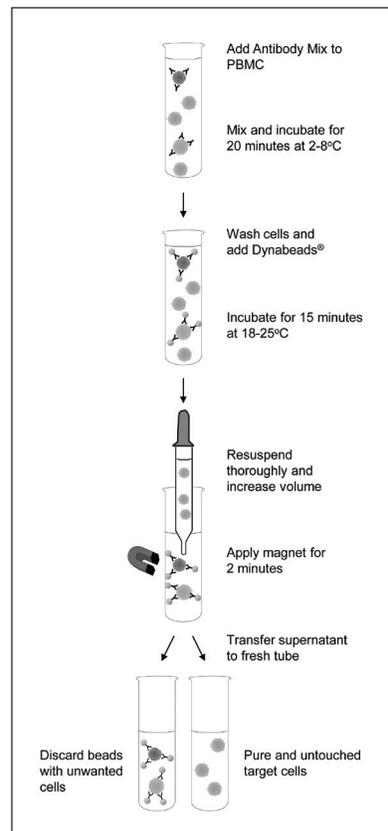


Figure 1: Isolation of untouched B Cells.

## Required Materials

- Magnet (DynaMag™) See [www.lifetechnologies.com/magnets](http://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<sup>2+</sup> and Mg<sup>2+</sup> 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](http://www.axis-shield-poc.com)).

## General Guidelines

- Visit [www.lifetechnologies.com/samplepreparation](http://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.
- Keep the buffers cold.

## 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 resuspend.
- Place the tube in a magnet for 1 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 PBMC suspension according to “General Guidelines”. Resuspend the cells at  $1 \times 10^8$  cells/mL in Isolation Buffer.

## Isolate Human B Cells

This protocol is based on  $5 \times 10^7$  PBMC, but is directly scalable from  $1 \times 10^7$  to  $5 \times 10^8$  cells, see Table 1.

1. Transfer 500  $\mu$ L ( $5 \times 10^7$ ) PBMC in Isolation Buffer to a tube.
2. Add 100  $\mu$ L of Antibody Mix.
3. Mix well and incubate for 20 min at 2°C to 8°C.
4. Wash the cells by adding 4 mL Isolation Buffer. Mix well by tilting the tube several times and centrifuge at  $350 \times g$  for 8 min at 2°C to 8°C. Discard the supernatant.
5. Resuspend the cells in 500  $\mu$ L Isolation Buffer.
6. Add 500  $\mu$ L pre-washed Dynabeads®.
7. Incubate for 15 min at 18°C to 25°C with gentle tilting and rotation.
8. Add 4 mL Isolation Buffer. (When working with lower cell volumes, never use less than 1 mL Isolation Buffer).
9. Resuspend the bead-bound cells thoroughly by pipetting >10 times using a pipette with a narrow tip opening. Avoid foaming.
10. Place the tube in the magnet for 2 min. Transfer the supernatant containing the untouched human B cells, to a new larger tube.
11. Add 4 mL Isolation Buffer to the tube containing the Dynabeads® and resuspend the bead-bound cells by pipetting as described in step 9.
12. Place the tube in the magnet for 2 min.
13. Combine the two supernatants.
14. *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 human B cells. This protocol is scalable from  $1 \times 10^7$  to  $5 \times 10^8$  PBMC.

Step	Step description	Volumes per $5 \times 10^7$ PBMC	Volumes per $2 \times 10^8$ PBMC
	Recommended tube size	15 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Cell volume	500 $\mu$ L	5 mL
2	Antibody Mix	100 $\mu$ L	1 mL
4*	Wash cells (Isolation Buffer)	~4 mL	~40 mL
5	Resuspend cells (Isolation Buffer)	500 $\mu$ L	5 mL
6**	Depletion Dynabeads®	500 $\mu$ L	5 mL
8-11*	Increase volume (Isolation Buffer)	2 $\times$ ~4 mL	2 $\times$ ~40 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™ SA Dynabeads® are uniform, superparamagnetic polystyrene beads (1.0  $\mu$ m diameter) coated with streptavidin (SA). The Antibody Mix contains biotinylated mouse IgG antibodies for CD2, CD14, CD16 (specific for CD16a and CD16b), CD36, CD43 and CD235a (Glycophorin A).

## Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
Dynabeads® Human T-Activator CD3/CD28	11131D
Dynabeads® Human T-Activator CD3/CD2/CD137	11162D
HulaMixer® Sample Mixer	15920D
Phosphate Buffered Saline	10010-023

**REF** on labels is the symbol for catalog number.

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