

DETACHaBEAD® CD19

Catalog no. 12506D

Store at 2°C to 8°C

Rev. Date: March 2012 (Rev. 004)

Product Contents

Product contents	Volume
DETACHaBEAD® CD19	5 mL

Kit capacity

1 mL of DETACHaBEAD® CD19 is sufficient to release cells from 2.5 mL Dynabeads® CD19 Pan B.

DETACHaBEAD® CD19 is polyclonal antibodies, supplied in 0.15 M phosphate buffered saline (PBS) pH 7.4.

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

Product Description

DETACHaBEAD® CD19 is intended for release of human CD19⁺ B cells from beads after positive isolation with Dynabeads® CD19 Pan B (not supplied). The released CD19⁺ cells are pure, viable, not activated by the release-step and have no beads or primary antibody bound to the surface. Release of beads from the cells using beads provides a fast and reliable method of positive isolation of human CD19⁺ B cells isolated from whole blood, buffy coat or mononuclear cells (MNC).

After positive isolation the DETACHaBEAD® CD19 is then added to the bead-bound cells, and will release the beads and primary antibody from the human CD19⁺ B cells. Following a short incubation period, the released beads are removed by magnetic separation and the positively selected cells collected. The isolated cells are pure, viable and are not activated by the isolation or release procedure.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes, (e.g. HulaMixer® Sample Mixer).
- Cell culture media, e.g. RPMI 1640 with 1% Fetal Bovine Serum (FBS).
- Dynabeads® CD19 Pan B.

General Guidelines

- Never resuspend the positively isolated cells in less than 100 µL cell culture media, even if the starting sample is less than 1 × 10⁷ MNC or 1 mL blood.
- The volume of beads used relates to the volume of beads used for cell isolation; never use less than 10 µL beads per 25 µL beads used for positive isolation. The amount of beads used can be directly scaled up.
- Incubation at 2°C to 8°C will significantly reduce the number of released cells. Incubating at 37°C will not increase release efficiency.
- It is important to remove the beads after release, to avoid background staining if using a flow cytometer.
- Due to the high protein concentration it is not unusual to see some precipitation in the DETACHaBEAD® CD19 component. This does not influence on product performance or quality.

Protocol

Prepare Sample

- Perform positive cell isolation with Dynabeads® CD19 Pan B according to that protocol.
- Resuspend the bead-bound cells in 250 µL cell culture media per 25 µL Dynabeads® CD19 Pan B.

Release Human CD19⁺ B Cells

The amount of DETACHaBEAD® needed to obtain optimal release correlates with the number of beads used. This protocol is based on an initial positive cell isolation step from a starting sample of 2.5 × 10⁷ cells using 25 µL beads resuspended in 250 µL cell medium. Never use less than 10 µL DETACHaBEAD® even when working with less than 25 µL beads. When working with >25 µL beads, scale up all volumes accordingly, as shown in Table 1.

1. Add 10 µL DETACHaBEAD® to the cell sample (250 µL).
2. Incubate for 45 min at room temperature with gentle mixing.
3. To enhance the release of cells, pipette the solution a few times before placing the tube in a magnet for 1 min.
4. Transfer the supernatant containing released cells to a fresh tube.
5. To obtain residual cells, wash the beads 2–3 times in 500 µL cell culture media and collect the supernatant.
6. Wash released cells thoroughly by resuspending the cells in a total volume of 10 mL cell culture media and centrifuge for 6 min at 400 × g to remove DETACHaBEAD®.
7. Resuspend the cells in cell culture media or other media and use in downstream application.

The isolated CD19⁺ cells are pure, viable, and are free from antibody or beads bound to the surface.

Table 1: Volumes for release of human CD19⁺ B cells.

Step	Step description	Volumes per 25 µL beads	Volumes per 250 µL beads
	Recommended tube size	2 mL	5 mL
	Recommended magnet	DynaMag™-2	DynaMag™-5
1	Cell volume	250 µL	2.5 mL
1	DETACHaBEAD® volume	10 µL	100 µL
5	Wash cells (cell culture media)	3 × 500 µL	3 × ~4 mL
6*	Remove DETACHaBEAD®	~10 mL	~15 mL

* Transfer the cells to a larger tube and fill it up with cell culture media to avoid background staining in the flow analysis.

Description of Materials

DETACHaBEAD® CD19 contains polyclonal anti-Fab antibodies specific for the CD19 antibody on the Dynabeads® CD19 Pan B. No cross-reaction with human IgG, IgM or IgA has been detected in quantitative ELISA tests.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Dynabeads® CD19 Pan B	11143D

REF on labels is the symbol for catalog number.

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