Miltenyi Biotec

Regulatory B Cell Isolation Kit

mouse

For 2×10^9 total cells,
up to 20 separations

Order no. 130-095-873

Miltenyi Biotec

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1. Description

Components

2 mL Regulatory B Cell Biotin-Antibody Cocktail, mouse:
Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against CD4, CD11c, CD49b, CD90.2, Gr-1, and Ter119.

2×2 mL Anti-Biotin MicroBeads:
MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).

1 mL Regulatory B Cell Catch Reagent, mouse:
anti-IL-10 monoclonal antibody (rat IgG2b) conjugated to CD45-specific monoclonal antibody (rat IgG2b).

1 mL Regulatory B Cell Detection Antibody (PE), mouse:
anti-IL-10 monoclonal antibody (rat IgG1) conjugated to PE (R-phycoerythrin).

2 mL Anti-PE MicroBeads:
MicroBeads conjugated to monoclonal anti-PE antibody (isotype: mouse IgG1).

Capacity

For 2×10^9 total cells, up to 20 separations.

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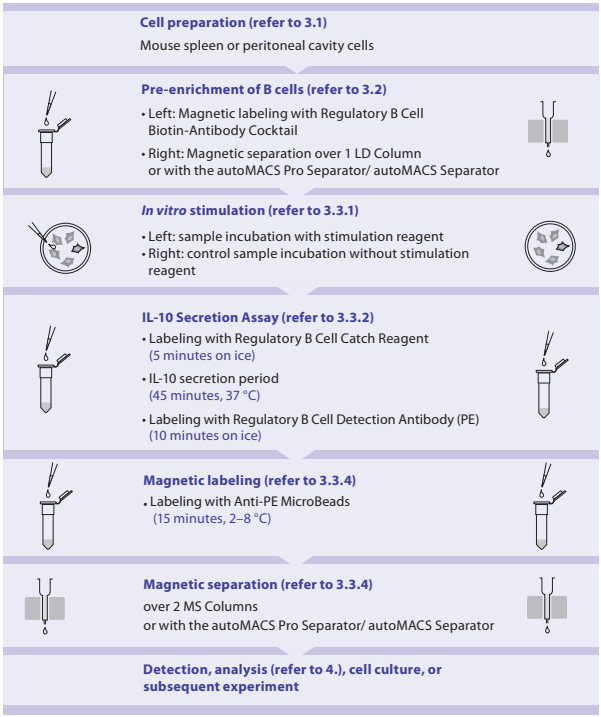
Product format All components are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

The isolation of regulatory B cells is performed in three steps.
First, the B cells are pre-enriched by depletion of non-B cells.
Second, the pre-enriched B cells are stimulated for 5 hours in culture with, e.g., LPS, PMA, and ionomycin to induce IL-10 secretion.
Third, the viable IL-10–producing cells are specifically isolated by using the Cytokine Secretion Assay technology.
Therefore, an IL-10–specific **Catch Reagent** is attached to the cell surface. The cells are incubated for a short time at 37 °C to allow for cytokine secretion. The secreted IL-10 binds to the Regulatory B Cell Catch Reagent on the cytokine-secreting cells. These cells are subsequently labeled with a second IL-10–specific antibody, the **Regulatory B Cell Detection Antibody** conjugated to PE for sensitive detection by flow cytometry. The IL-10–secreting cells can now be magnetically labeled with **Anti-PE MicroBeads** and enriched over a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled cells are retained within the column. The unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained IL-10–secreting cells can be eluted as positively selected cell fraction. The cells can now be used for cell culture or analysis. Since viable cells are analyzed, non-specific background can be minimized by dead cell exclusion. This provides highest sensitivity of analysis.

1.2 Protocol overview



1.3 Background information

Immunological tolerance exemplifies the capacity of the immune system to down-modulate immune responses. B cells are generally considered to positively regulate immune responses by producing Ag-specific antibodies and by helping to induce optimal $CD4^+$ T cell activation. However, B cells and specific B cell subsets can also negatively regulate immune responses in mice, validating the existence of regulatory B cells. The regulatory function is directly accomplished by the production of the cytokine IL-10. Autoimmunity and inflammation are controlled in part by these regulatory B cells.
The Regulatory B Cell Isolation Kit is designed for the isolation, detection, and analysis of viable IL-10–secreting murine B cells.[1-3]

1.4 Applications

- Detection and enrichment of viable IL-10–secreting regulatory B cells for phenotyping and functional characterization.

1.5 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Culture medium, e.g., RPMI 1640 with stable glutamin (# 130-091-439), containing 10% FBS, 200 µg/mL penicillin, 200 U/mL streptomycin, and 5×10^{-5} M 2-ME.
- Cell stimulation reagents, for example, LPS, PMA, and ionomycin, or CD40 pure – functional grade, mouse (# 130-093-023, # 130-093-022).
- Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD19 Antibody, anti-mouse, APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

- MACS Columns and MACS Separators: Choose the appropriate MACS Column and MACS Separator according to the number of total cells and the number of labeled cells.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion of non-B cells			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS
Enrichment of regulatory B cells			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- Refrigerated centrifuge (2–8 °C).
- Rotation device for tubes: MACSmix™ Tube Rotator (# 130-090-753).
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

2. Experimental setup

2.1 Controls

Negative control

For accurate detection of stimulated IL-10–secreting regulatory B cells, a negative control sample should always be included. This will provide information about B cells enriched unspecifically over binding phycoerythrin. The control sample should be treated exactly the same way as the stimulated sample except for the addition of the stimulation reagent. Cells isolated from an IL-10 knockout mouse can also be used as a negative control.

2.2 Counterstaining of regulatory B cells

The regulatory B cells are stained with PE-conjugated Regulatory B Cell Detection Antibodies. To identify cells of interest, counterstaining for B cells with, for example, CD19-APC, mouse (# 130-092-039) is important.

▲ Do not use tandem conjugates of phycoerythrin, such as Cy™-Chrome, PE-Cy5, ECD, or PC5. They may also be recognized by the Anti-PE MicroBeads.

▲ The samples should be stained with propidium iodide (PI), for example with Propidium Iodide Solution (# 130-093-233), or 7-AAD prior to acquisition, to exclude dead cells from analysis. This will reduce non-specific background staining and increase sensitivity.

3. Protocol

3.1 Cell preparation

Prepare a single-cell suspension under sterile conditions from mouse spleen using manual methods or the gentleMACS™ Dissociator.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

3.2 Pre-enrichment of B cells

3.2.1 Magnetic labeling of non-B cells

▲ Work under sterile conditions.

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^8 total cells. When working with fewer than 10^8 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^8 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

▲ (Optional) Block Fc receptors. For details refer to the FcR Blocking Reagent, mouse (# 130-092-575) data sheet.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of Regulatory B Cell Biotin-Antibody Cocktail per 10^8 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 300 µL of buffer and 200 µL of Anti-Biotin MicroBeads per 10^8 total cells.
7. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
8. Proceed to magnetic separation (3.2.2).

3.2.2 Magnetic separation: Depletion of non-B cells

▲ Work under sterile conditions.

▲ Always wait until the column reservoir is empty before proceeding to the next step.



Depletion using LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; this is the unlabeled pre-enriched B cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 3 mL of buffer onto the column. Immediately flush out the magnetically labeled non-B cells by firmly pushing the plunger into the column.
6. Proceed to 3.3 for the stimulation of B cells.



Depletion with the autoMACS® Pro Separator or the autoMACS® Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Depletion: Depl05
   Collect negative fraction in row B of the tube rack. This fraction represents the pre-enriched B cells.
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-B cells.
5. Proceed to 3.3 for the stimulation of B cells.

Magnetic separation with the autoMACS® Separator

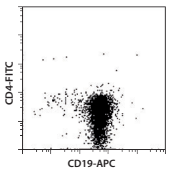
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Depletion: Depl05
   Collect negative fraction from outlet port neg1. This fraction represents the pre-enriched B cells.
4. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-B cells.
5. Proceed to 3.3 for the stimulation of B cells.

3.2.3 Example of pre-enrichment of B cells

Regulatory B cells were isolated from mouse spleen using the Regulatory B Cell Biotin-Antibody Cocktail, Anti-Biotin MicroBeads, an LD Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD19-APC and CD4-FITC and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Pre-enriched B cells



3.3 Enrichment of regulatory B cells

▲ Always include a negative control in the experiment (refer to 2.1).



3.3.1 *In vitro* stimulation

1. Determine cell number.
2. Wash cells by adding culture medium, centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cells in culture medium at 2.5×10^6 cells/mL and 1.25×10^6 cells/cm², e.g., 5×10^6 B cells in 2 mL medium/well of a 12-well plate.
4. Add stimulation reagent, e.g., 10 µg/mL LPS *E. coli* serotype 0111: B4 for 5–48 hours at 37 °C, 5% CO_2 and add 50 ng/mL PMA and 500 ng/mL ionomycin for the last 5 hours of stimulation.

   For comparison of different experiments, the stimulation time should always be the same (refer to 2.1).
5. Collect cells carefully by pipetting up and down. Rinse the dish with cold buffer. Check microscopically for any remaining cells, if necessary, rinse the dish again.

3.3.2 IL-10 Secretion Assay

General considerations

▲ For each test with 10^7 total cells, prepare:
100 mL of **cold buffer** (2–8 °C)
100 µL of **cold medium** (2–8 °C)
10 mL of **warm medium** (37 °C).

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling (exception: warm medium during secretion period).

▲ Volumes given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ Do not remove supernatant by decanting. This will lead to cell loss and incorrect incubation volumes. Aspirate supernatant.

▲ Dead cells may bind non-specifically to antibodies. Therefore, when working with cell preparations containing large amounts of dead cells, they should be removed before starting the IL-10 Secretion Assay, for example, by densitiy gradient centrifugation or by using the Dead Cell Removal Kit (# 130-090-101).



Labeling cells with Regulatory B Cell Catch Reagent

1. Determine cell number.
2. Use 10^7 total cells in a 15 mL closable tube per sample.
3. Wash cells by adding 10 mL of **cold buffer**, centrifuge at 300×g for 10 minutes at 2–8 °C, aspirate supernatant completely. Repeat washing step.
4. (Optional) Block Fc receptors. For details refer to the FcR Blocking Reagent, mouse (# 130-092-575) data sheet.
5. Resuspend cell pellet in 90 µL of **cold medium** per 10^7 total cells.
6. Add 10 µL of **Regulatory B Cell Catch Reagent** per 10^7 total cells, mix well and incubate for 5 minutes on ice.



IL-10 secretion period

1. Add 10 mL **warm medium** (37 °C) per 10^7 total cells.
2. Incubate cells in closed tube for 45 minutes at 37 °C under slow continuous rotation using the MACSmix Tube Rotator (# 130-090-753), or turn tube every 5 minutes to resuspend settled cells.

   ▲ **Note:** During this step it is crucial to prevent contact of cells to avoid cross contamination with cytokines.



Labeling cells with Regulatory B Cell Detection Antibody (PE)

1. Fill the tube with **cold buffer**.
2. Put the tube on ice for 5 minutes.

   ▲ **Note:** Cells must be cooled to prevent unspecific labeling of cells with IL-10.
3. Centrifuge cells at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.

   ▲ **Note:** If the volume of the cell suspension was higher than the volume of the added buffer, then repeat the wash step.
4. Resuspend cell pellet in 90 µL of **cold buffer** per 10^7 total cells.
5. Add 10 µL of **Regulatory B Cell Detection Antibody (PE)** per 10^7 total cells.
6. (Optional) Add staining antibodies, e.g., 10 µL of CD19-APC.

   ▲ **Note:** Do not use tandem conjugates of phycoerythrin.
7. Mix well and incubate for 10 minutes on ice.
8. Add 10 mL of **cold buffer** and centrifuge at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.
9. Proceed to magnetic labeling (3.3.3).

3.3.3 Magnetic labeling of IL-10–secreting B cells

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.



Magnetic labeling with Anti-PE MicroBeads

1. Resuspend cell pellet in 80 µL of **cold buffer** per 10^7 total cells.
2. Add 20 µL of **Anti-PE MicroBeads** per 10^7 total cells.

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3. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
4. Wash cells by adding 10 mL of **cold buffer** per 10^7 total cells and centrifuge at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.
5. Resuspend cell pellet in 500 µL of **cold buffer**. For higher cell numbers than 5×10^7 use a dilution of 10^8 cells/mL.
6. Proceed to magnetic separation (3.3.4).

3.3.4 Magnetic separation: Enrichment of IL-10–secreting B cells



Magnetic separation using MS or LS Columns

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of labeled cells. For details refer to the table in section 1.5.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

▲ Always perform two consecutive column runs to achieve best results.

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL

3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. To increase the purity of IL-10–secreting cells, the eluted fraction must be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

   ▲ **Note:** For subsequent cell culture, the cells can also be eluted with medium. If part of the cells are analyzed by flow cytometry, the medium should not contain phenol red.

8. Proceed to analysis (refer to section 4), cell culture, or other subsequent experiment.

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Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Positive selection: Posseld
   Collect positive fraction in row C of the tube rack.
4. Proceed to analysis (refer to section 4), cell culture, or other subsequent experiment.

Magnetic separation with the autoMACS® Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose the following program:

   Positive selection: Posseld
   Collect positive fraction from outlet port pos2.
4. Proceed to analysis (refer to section 4), cell culture, or other subsequent experiment.

4. Example of detection and analysis of regulatory B cells

▲ Add propidium iodide (PI) or 7-AAD to a final concentration of 0.5 µg/mL just prior to acquisition to exclude dead cells from flow cytometric analysis. Incubating with PI for longer periods will affect the viability of the cells.
Do not fix the cells when using PI or 7-AAD.

▲ For optimized sensitivity, an appropriate number of viable cells has to be acquired from the antigen stimulated sample as well as from the control sample.

To illustrate the analysis, we describe the detection of regulatory B cells using the Regulatory B Cell Isolation Kit, mouse. The detailed description, including how to set gates, should serve as a model for the analysis of your own sample.

1. B cells were pre-enriched from BALB/c mouse spleen and have been incubated for 5 hours with and without LPS, PMA, and ionomycin.
2. The isolation of viable IL-10–producing B cells was performed by using the principle of the Cytokine Secretion Assay on the stimulated and the unstimulated sample.
3. Counterstaining of regulatory B cells was performed using CD19-APC.
4. Dead cells were stained with PI, which was added just prior to flow cytometric analysis to a final concentration of 0.5 µg/mL.
5. 10,000 viable cells of the fractions before enrichment and the complete enriched fractions were acquired by flow cytometry using the MACSQuant® Analyzer, from the stimulated as well as from the unstimulated samples.
6. A lymphocyte gate based on forward scatter (FSC) and side scatter (SSC) properties was activated prior to further gating to exclude debris (A).
7. Dead cells were excluded according to PI-staining (B).

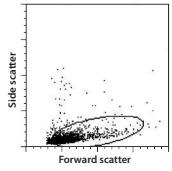
   ▲ **Note:** The dead cell exclusion is crucial for the analysis for regulatory B cells, as dead cells may bind non-specifically to MicroBeads or antibodies. This could lead to false positive events.
8. Analysis of secreted IL-10 (PE) versus CD19-APC staining of viable lymphocytes is displayed (C).

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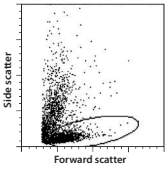
4. Example of detection and analysis of regulatory B cells

A) Lymphocyte gate

Before enrichment

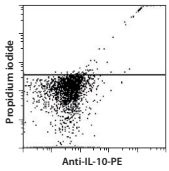


After enrichment

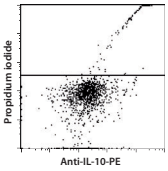


B) Dead cell exclusion

Before enrichment

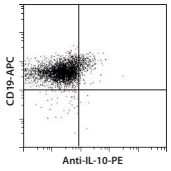


After enrichment



C) Stimulated sample

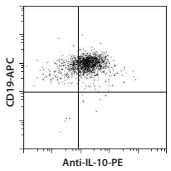
Before enrichment



10.6%–5.1% (negative control) = 5.5% of the total B cell population secrete IL-10 (see formula below).

$$\%\ \text{IL-10}^+\ \text{cells among CD19}^+ = \frac{\#\ \text{of IL-10}^+\text{CD19}^+\ \text{cells in the analyzed sample}}{\#\ \text{of total CD19}^+\ \text{cells in the analyzed sample}} \times 100$$

After enrichment



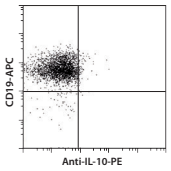
The IL-10–secreting B cells have been enriched to 87%.
28,300–2000 (negative control) = 26,300 IL-10$^+$ B cells were enriched from 10^6 CD19$^+$ cells (= 2.8%–0.2% (negative control) = 2.6%; see formula below).

$$\%\ \text{IL-10}^+\ \text{cells among CD19}^+ = \frac{\text{abs.}\ \#\ \text{of IL-10}^+\text{CD19}^+\ \text{cells in the enriched fraction}}{\text{abs.}\ \#\ \text{of total CD19}^+\text{cells before enrichment}} \times 100$$

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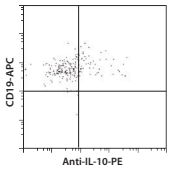
Unstimulated control sample

Before enrichment



5.1% of the total B cell population secrete IL-10.

After enrichment



2000 IL-10$^+$ B cells was enriched from 10^6 CD19$^+$cells (0.2%).

5. References

1. Mizoguchi, A. and Bhan, A.K. (2006) A case for regulatory B cells. J. Immunol. 176: 705–710.
2. Yanaba, K. *et al.* (2008) A regulatory B cell subset with a unique CD1d^{hi}CD5$^+$ phenotype controls T cell–dependent inflammatory responses. Immunity 28: 639–650.
3. DiLillo, D.J. *et al.* (2010) B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer. Ann. N Y Acad. Sci. 1183: 38–57.

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Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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