Miltenyi Biotec

SARS-CoV-2 Spike B Cell MicroBead Kit
human

Order no. 130-128-031

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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.

1. Description

This product is for research use only.

Components	**25 µg Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin – research grade** **60 µL Streptavidin, PE:** Streptavidin conjugated to PE. **2 mL Anti-PE MicroBeads UltraPure:** MicroBeads conjugated to monoclonal anti-PE antibodies (isotype: mouse IgG1).
Capacity	For 2×10^8 total B cells, up to 40 separations.
Product format	Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin is supplied lyophilized from a filtered (0.2 µm) buffer solution. Streptavidin, PE and Anti-PE MicroBeads UltraPure are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin at –20 °C. Upon reconstitution aliquots should be stored at –20 °C or below. Avoid repeated freeze-thaw cycles. Store Anti-PE MicroBeads UltraPure and Streptavidin, PE protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial labels.

1.1 Principle of the MACS Separation

First, the $CD19^+$ cells are magnetically labeled with spike-tetramer-PE (tetramer formed from Streptavidin, PE and Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin), washed, and labeled with Anti-PE MicroBeads UltraPure. After washing, the cell suspension is loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled antigen-specific B cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of antigen specific B cells. After removing the column from the magnetic field, the magnetically retained antigen-specific B cells cells can be eluted as the positively selected cell fraction.

1.2 Background information

B cells, also known as B lymphocytes, express, dependent on their maturation stage, the antigen-specific B cell receptor (BCR) on their surface or secrete antigen-specific antibodies. They are part of the adaptive immune system and are crucial to mount a humoral, long lasting sterile immunity. B cells also play a role as professional antigen-presenting cells and secrete cytokines. They circulate through the body via the peripheral blood and account for 2–10% of all lymphocytes. Mature B cells in blood express the pan B cell marker CD19 and most mature B cells except plasma cells express CD20 and CD22. Additionally, different subsets of memory B cells and plasma cells can be identified based on their expression of Ig isotypes (IgM, IgD, IgG, IgA, IgE)[1]. Antigen-specific B cells usually occur at a frequency less than 0.05% for a specific antigen, but their number can vary depending on the phase and type of antigen or immunization [2,3,4].

The quantitative and qualitative analysis of antigen-specific B cells specifically recognizing and reacting towards a defined antigen provide important information to understand their function in various immunological situations. The presence of these cells indicate that an individual is mounting an adaptive response to the specific, infective pathogen or to an immunization containing that specific antigen.[5,6] Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) induces an adaptive immune response leading to SARS-CoV-2–specific immunoglobulin and SARS-CoV-2–specific memory B and memory T cells with variable persistency and antiviral efficacy.[5,6,7,8] Analysis and enrichment of antigen-specific B cells can contribute to the understanding of the role of cellular and humoral response and thus to the protection from SARS-CoV-2 infection after a previous infection or immunization.[9,10]

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140-006-125.01

1.3 Applications

- Positive selection of SARS-CoV-2–specific B cells from pre-enriched PBMCs. The several-fold enriched antigen-specific B cells could be used for:
  - expansion in culture,
  - molecular analysis, e.g. B cell receptor (BCR) cloning and sequencing,
  - single clone culture.

1.4 Reagent and instrument requirements

- REAlease CD19 MicroBead Kit, human (# 130-117-034) for the enrichment of B cells prior the labeling with spike-tetramer-PE to obtain an optimal performance.
- Phosphate-buffered saline (PBS) without azide, protein, or other amine-containing compound, pH 7.2.
- PEB 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.
- MACS Columns and MACS Separators: SARS-CoV-2–specific B cells can be enriched by using MS Columns.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD19 Antibody, anti-human (clone LT19, isotype: mouse IgG1κ). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.
- (Optional) 8-Color Immunophenotyping Kit (# 130-120-640) for quality control of the PBMC samples.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

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

2.2 Spike-tetramer-PE preparation

1. Resuspend Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin in deionized, sterile-filtered water to a final concentration of 0.1 mg/mL (e.g. use 250 µL water for 25 µg protein).

   ▲ **Note:** To avoid repeated freeze-thaw cycles prepare working aliquots from the stock solution of the Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin. Store the working aliquots at –70 °C. Once thawed the protein can be stored for up to three days at 4 °C.

2. For each separation (5×10^6 B cells) prepare the spike-tetramer-PE by mixing 6 µL Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin, 1.2 µL Streptavidin, PE, and 2.8 µL PEB buffer. Incubate for 15 minutes at room temperature.

   ▲ **Note:** Always freshly prepare the spike-tetramer solutions before use. They can be stored for up to 24 hours at 2–8 °C, protected from light.

   ▲ **Note:** When working with less than 5×10^6 B cells, use the same volumes as indicated. When working with more than 5×10^6 B cells, scale up all reagent volumes accordingly.

2.3 Enrichment of SARS-CoV–specific B cells

1. Enrich B cells from a single-cell suspension of PBMCs using the REAlease CD19 MicroBead Kit according to the data sheet of the kit including removal of the REAlease Complex.
2. Proceed with magnetic labeling (2.4).



2.4 Magnetic labeling

▲ 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 5×10^6 total B cells. When working with fewer than 5×10^6 B 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 10^7 total B 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.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 100 µL of PEB buffer per 5×10^6 total B cells. Add 10 µL of spike-tetramer-PE and incubate for 30 minutes at 4 °C.
4. Wash cells by adding 1–2 mL of PEB buffer per 5×10^6 total B cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

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5. Resuspend cell pellet in 160 µL of PEB buffer per 5×10^6 total B cells.
6. Add 40 µL of Anti-PE MicroBeads UltraPure per 5×10^6 total B cells.
7. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
8. Wash cells by adding 1–2 mL of buffer per 5×10^6 total B cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^7 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.5).

2.5 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of B cells. For details refer to the table in section 1.4.

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

Magnetic separation with MS Columns

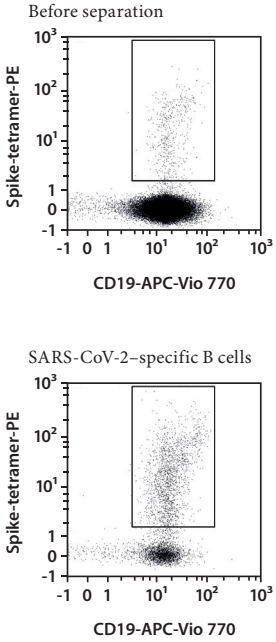
1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

3. Example of a separation using the SARS-CoV-2 Spike B Cell MicroBead Kit

B cells were enriched from human PBMCs from convalescent individuals using the REAlease CD19 MicroBead Kit, including the removal of the REAlease Complex. Then, SARS-CoV-2 spike-specific B cells were isolated using the SARS-CoV-2 Spike B Cell MicroBead Kit, human, an MS Column, and a MiniMACS™ Separator.

Cells were fluorescently stained with spike-tetramer-PE and CD19-APC-Vio® 770 and analyzed by flow cytometry using the MACSQuant® Analyzer 10. Cell debris and dead cells were excluded from the analysis based on scatter signals and 7-AAD fluorescence.



4. References

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