Miltenyi Biotec

SARS-CoV-2 Spike B Cell Analysis Kit, anti-human

Order no. 130-128-022

Contents

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

60 µL Streptavidin, PE-Vio® 770

60 µL CD19 Antibody, anti-human, APC-Vio 770 (clone: LT19, isotype: mouse IgG1κ)

60 µL CD27 Antibody, anti-human, Vio Bright FITC (clone: M-T271, isotype: mouse IgG1κ)

60 µL IgG Antibody, anti-human, VioBlue® (clone: IS11-3B2.2.3, isotype: mouse IgG1κ)

60 µL IgA Antibody, anti-human, VioGreen™ (clone: IS11-8E10, isotype: mouse IgG1κ)

60 µL IgM Antibody, anti-human, APC (clone: PJ2-22H3, isotype: mouse IgG1)

1 mL 7-AAD Staining Solution

Capacity

25 tests for up to 1.25×10^8 total B cells or 2.5×10^8 peripheral blood mononuclear cells (PBMCs).

Product format

Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide. The ready-to-use 7-AAD Staining Solution is supplied in phosphate-buffered saline, pH 7.2, at a concentration of 52.5 µg/mL. Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin is supplied lyophilized from a filtered (0.2 µm) buffer solution.

Storage

Store antibodies and 7-AAD Staining Solution protected from light at 2–8 °C. Do not freeze. 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. The expiration dates are indicated on the vial labels.

1.1 Principle

The SARS-CoV-2 B Cell Analysis Kit, anti-human has been developed for the fast and easy detection of SARS-CoV-2–specific B cells by binding of SARS-CoV-2–specific proteins to the respective antigen-specific B cell receptor (BCR) on B cells circulating in the peripheral blood of individuals who developed a B cell response against SARS-CoV-2 proteins. The kit contains SARS-CoV-2 recombinant proteins and Streptavidin conjugates to prepare spike-tetramer solutions (tetramers formed from Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin with Streptavidin, PE and PE-Vio 770, respectively). Fluorochrome-conjugated antibodies in the kit allow the identification and phenotyping of B cells (memory B cells and isotypes). 7-AAD Staining Solution is used for the exclusion of dead and apoptotic cells. The optimized flow panel and protocol ensures an easy and efficient analysis of SARS-CoV-2–specific B cells.

1.2 Background information

The quantitative and qualitative analysis of antigen-specific B cells specifically recognizing and reacting towards a defined antigen provides 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. Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) induces an adaptive immune response leading to SARS-CoV-2–specific immunoglobulins and SARS-CoV-2–specific memory B and memory T cells with variable persistency and antiviral efficacy. Analysis and enrichment of antigen-specific B cells can contribute to the understanding of the role of cellular and humoral responses and thus to the protection from SARS-CoV-2 infection after a previous infection or immunization.

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1.3 Applications

Identification and characterization of SARS-CoV-2–specific B cells circulating in peripheral blood of individuals who developed a B cell response against SARS-CoV-2 proteins:

- Sensitive and specific quantification of the numbers of antigen-specific B cells.
- Phenotyping of differentiation status and isotype (IgG, IgA, IgM) of SARS-CoV-2–specific B cells.
- Cell sorting of SARS-CoV-2–specific B cells.

1.4 Reagent and instrument requirements

- Phosphate-buffered saline (PBS), pH 7.2, without azide, protein, or other amine-containing compounds.
- 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).
- Flow cytometer equipped with a red (640 nm), a blue (488 nm), and a violet (405 nm) laser, e.g., MACSQuant® Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803).
- (Optional) REAlease® CD19 MicroBead Kit, human (# 130-117-034) for pre-enrichment of B cells.
- (Optional) StemMACS™ Cryo-Brew (# 130-109-558) for cryopreservation of B cells after pre-enrichment.
- (Optional) 8-Color Immunophenotyping Kit, anti-human, REAfinity (# 130-120-640) for quality control of the PBMC samples.
- (Optional) MACS Comp Bead Kit, anti-human Igκ (# 130-104-187) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocol

2.1 Reagent preparation

▲ Volumes given below are for up to 5×10^6 B cells or 10^7 PBMCs. When working with fewer 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 B cells, use twice the volume of all indicated reagent volumes and total volumes).

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 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 2–8 °C.

2. Prepare both spike-tetramer solutions by mixing Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin, PEB buffer, and Streptavidin, PE or PE-Vio 770 (50 µg/mL), respectively, as indicated in table 1. Incubate for 15 minutes at room temperature.

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

Spike-tetramer solution	Recombinant SARS-CoV-2 Spike-Prot (HEK)-Biotin	Streptavidin fluorochrome	PEB buffer
Spike-tetramer-PE	3 µL	0.6 µL Streptavidin, PE	1.4 µL
Spike-tetramer-PE-Vio 770	6 µL	1.2 µLStreptavidin, PE-Vio 770	2.8 µL

Table 1: Volumes for preparation of spike-tetramer solutions for one test with 5×10^6 B cells or 10^7 PBMCs. Note that handling of small volumes below 1 µL might cause imprecisions. Therefore, it is recommended to scale up reagents to prepare solution with bigger volumes.

3. Prepare the antibody staining cocktail: For each test mix 2 µL of each fluorochrome-conjugated antibody, 5 µL of 7-AAD Staining Solution, 5 µL of spike-tetramer-PE, and 10 µL of spike-tetramer-PE-Vio 770. Fill up to 100 µL with PEB buffer.

2.2 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.

2.3 Staining of SARS-CoV-2 spike–specific B cells

▲ $CD19^+$ cells can be enriched from single-cell suspension of PBMCs using the REAlease CD19 MicroBead Kit. Follow the instructions in the data sheet.

1. Resuspend 5×10^6 B cells or 10^7 PBMCs in 100 µL of antibody staining cocktail. Incubate for 30 minutes in the refrigerator (2–8 °C).
2. Wash cells by adding 1–2 mL of PEB buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in a suitable amount of PEB buffer and analyze by flow cytometry using a suited flow cytometer, e.g. the MACSQuant® Analyzer 16.

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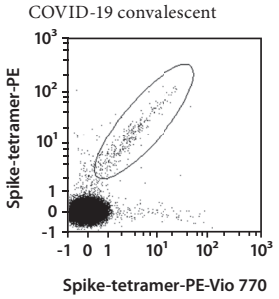
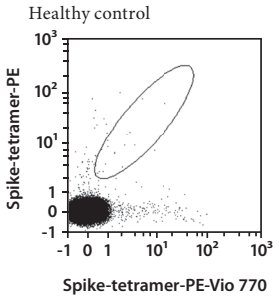
3. Example of immunofluorescent staining using the SARS-CoV-2 Spike B Cell Analysis Kit, anti-human

PBMCs of COVID-19 convalescent and healthy donors were obtained by density centrifugation. B cells were enriched using the REAlease CD19 MicroBead Kit, human. Cells were used fresh or cryopreserved in StemMACS Cryo-Brew.
Samples of label-free B cells were stained using the SARS-CoV-2 Spike B Cell Analysis Kit, human and analyzed by flow cytometry using the MACSQuant Analyzer 10. Cell debris, doublets, and dead cells were excluded from the analysis based on scatter signals and 7-AAD fluorescence. $CD19^+$ cells were gated and spike-specific B cells were detected using the double discrimination method.





Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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