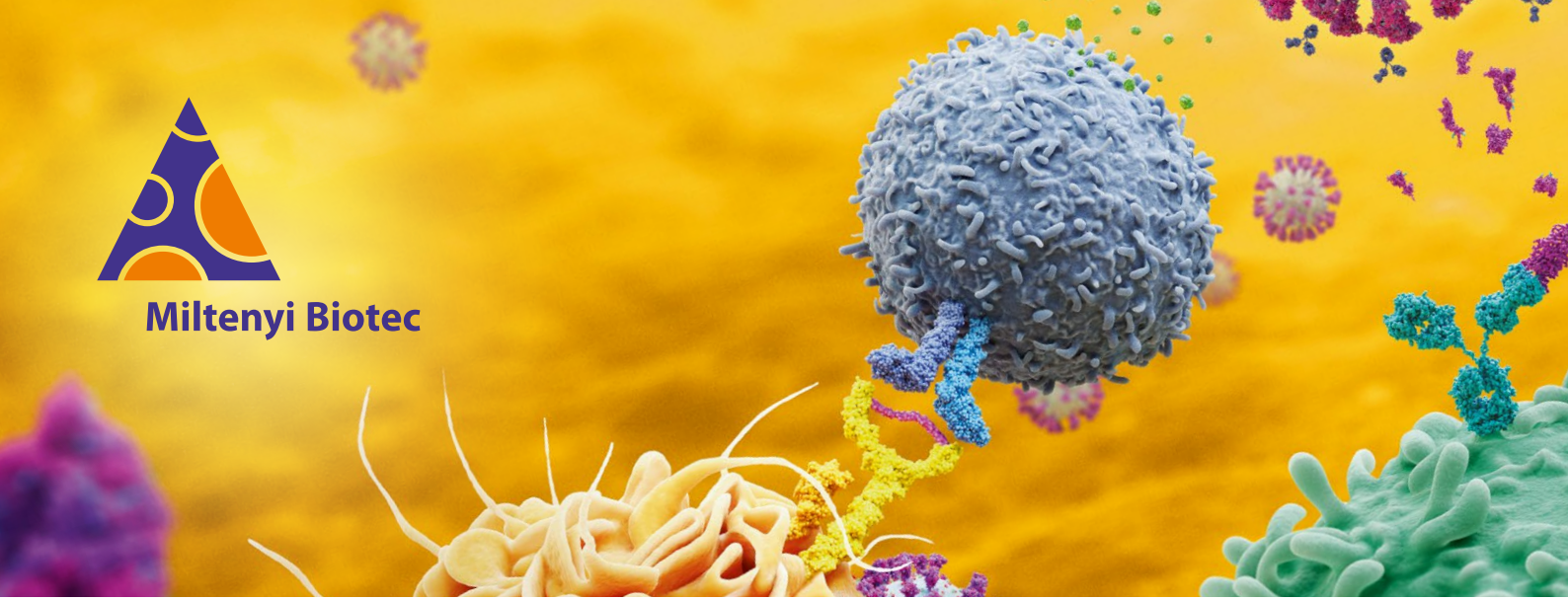

Miltenyi Biotec

MACSQuant® Tyto® Cell Sorter

Sorting of rare SARS-CoV-2-specific B cells

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Background

The need to safely sort infectious material has gained more attention since the outbreak of COVID-19. Sorting cells from patient samples to understand cellular processes and develop treatments, and vaccines is crucial. However, handling infectious material is often a challenge. How can we achieve safe cell sorting by protecting both, the operator and our samples? The concept of the MACSQuant Tyto considers this: The instrument works without fluidics since the whole sorting process takes place in a disposable, fully closed, and sterile cartridge (fig. 1). Thus, it represents the perfect solution for safe cell sorting. In addition, the MACSQuant Tyto does not produce aerosols and can be operated on a benchtop. Another challenge of cell sorting is to preserve the functionality of the cells. Delicate immune cells need to be sorted gently to sustain their functionality and viability. This is of particular interest, especially in the field of immunotherapy, where sample quality is important for proper downstream analysis on the path to discover new advanced therapies. Here we show that rare antigen-specific B cells from COVID-19 convalescent donors can be sorted on the MACSQuant Tyto without compromising their performance. Biological functionality of sorted B cells is tested in expansion and antibody secretion assays. Cells leave the input chamber and cross a filter system before entering the microchip through a microchannel, where they are interrogated by the three lasers (fig. 1). When a target cell (pink) is identified, a magnetic pulse emanating from the solenoid opens the microvalve, which then redirects the target cell into the positive collection chamber. In the default state the valve is closed, allowing non-target cells (blue and orange) to flow into the negative collection chamber.

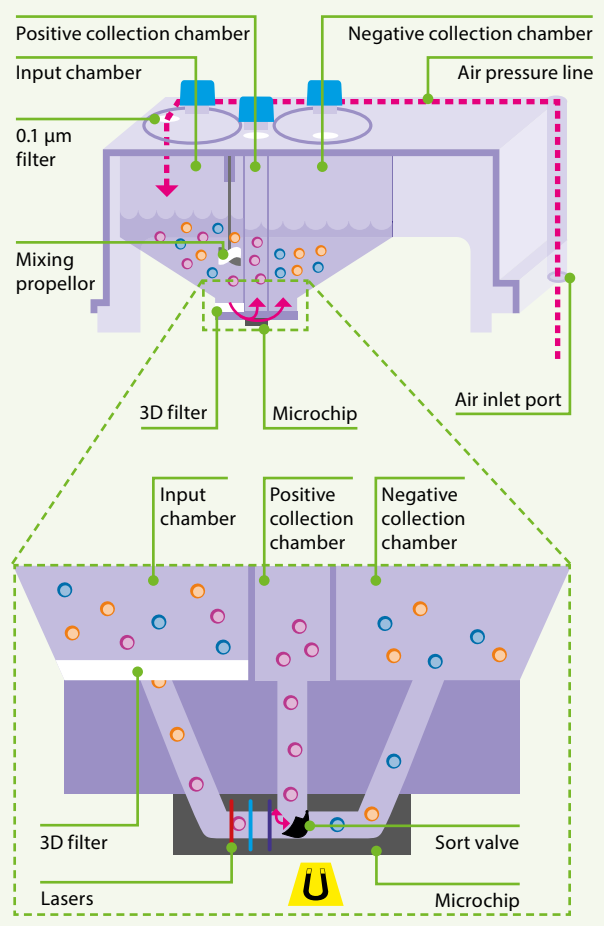

Figure 1: The sorting mechanism of the MACSQuant Tyto. Cells leave the input chamber and cross a filter system before entering the microchip through a microchannel, where they are interrogated by the three lasers. When a target cell (pink) is identified, a magnetic pulse emanating from the solenoid opens the microvalve, which then redirects the target cell into the positive collection chamber. In the default state the valve is closed, allowing non-target cells (blue and orange) to flow into the negative collection chamber.

Methods

Sample preparation and labeling of B cells
PBMCs were isolated using density gradient centrifugation from peripheral blood of plasma donors with high anti-SARS-CoV-2 antibody titers in the blood. Further, B cells were pre-enriched using the REAlease® CD19 MicroBead Kit (130-117-034).

To detect SARS-CoV-2-specific B cells, samples were stained with tetramers consisting of SARS-CoV-2 recombinant proteins conjugated to PE and PE-Vio® 770 using the SARS-CoV-2 Spike B Cell Analysis Kit, human (130-128-022) according to the datasheet.

Sorting of antigen-specific B cells
Spike-specific B cells were sorted on the MACSQuant Tyto. For this, 1–3×10^6 B cells were resuspended in 5 mL of MACSQuant Tyto Running buffer and loaded into a MACSQuant Tyto Cartridge HS, the high-speed version of the cartridge. It facilitates a significantly reduced sorting time while increasing sort performance through inertial focusing. The target cell frequency of spike-specific B cells was 0.1–0.3% in the starting material. The gating strategy started by gating on live B cells by using 7-AAD and CD19 (fig. 2), followed by choosing Spike-tetramer-positive cells. Finally, the sort gate is selected for IgG-positive cells. Alternatively, the Spike-tetramer-positive B cells from the gating step before can also be chosen as sort gate depending on whether only IgG-expressing or all antigen-specific B cells should be sorted. The sample was sorted at a flow rate of 8 mL/h.

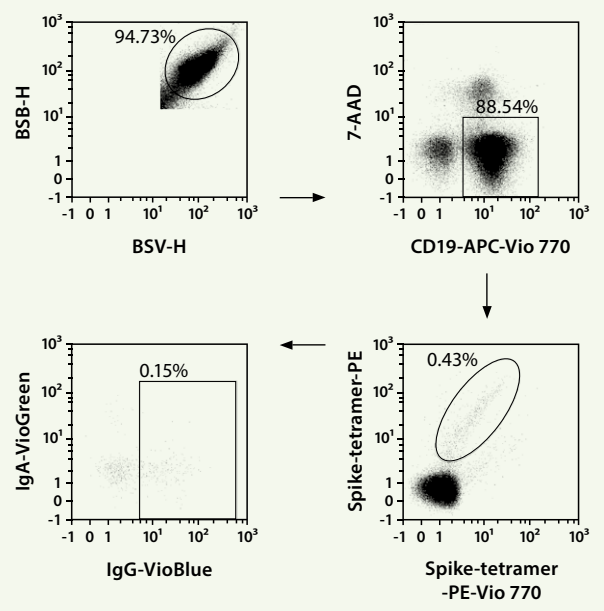

Figure 2: Gating strategy of spike-specific B cells on the MACSQuant Tyto. First, debris is gated out followed by gating on live B cells with the use of 7-AAD and CD19. Then, a gate is drawn around Spike-tetramer-positive cells and the hierarchy is finished by choosing a sort gate for IgG-positive cells.

Analysis of cell sorting
Analysis of the cells was performed by flow cytometry using the MACSQuant Analyzer 10 and the MACSQuantify™ Software. The he first gate focused on live cells using 7-AAD. Afterwards, CD19⁺ cells were gated and spike-specific B cells were detected using the SARS-CoV-2-specific tetramers conjugated to PE and PE-Vio 770. Finally, IgG-expressing SARS-CoV-2-specific B cells were defined as sort gate.

B cell expansion and SARS-CoV-2–specific antibody secretion
After sorting, B cells were expanded in culture in presence of CD40 ligand multimer and IL-4 using the B cell expansion kit (130-106-196) according to the following protocol availble on the homepage:

Microchip-based cell sorting and expansion of SARS-CoV-2–specific B cells using recombinant SARS-CoV-2 proteins and the MACSQuant® Tyto® Cell Sorter

After 14 days of culture, the B cell expansion rate was analyzed by comparing the number of cells on day 14 to the number of plated cells on day 0. Cell counting was performed using the MACSQuant Analyzer 10. Additionally, cell culture supernatants were harvested on day 7, 9 and 14 and were analyzed for secretion of anti-Spike-specific IgGs, measured on the MACSQuant 10 using SARS-CoV-2 recombinant proteins according to the following protocol available on the homepage:

Bead-based immunoassay for detection of patient-derived SARS-CoV-2 antibodies

Results

SARS-CoV-2-specific B cells were enriched from 0.10%–91.23% with the MACSQuant Tyto Cell Sorter. Moreover, the microchip-based gentle cell sorting principle allowed a viability of the sensitive antigen-specific B cells of >98% and full downstream compatibility (fig. 3).

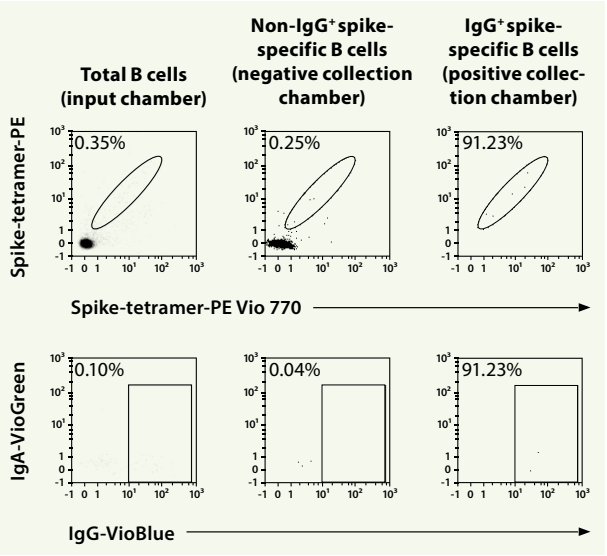

Figure 3: Flow cytometry analysis of cells obtained from the input, the negative, and the positive collection chamber of the MACSQuant Tyto Cartrdige HS. PBMCs were pre-enriched for B cells, labeled using the SARS-CoV-2 Spike B Cell Analysis Kit, human and Spike⁺IgG⁺ B cells were sorted on the MACSQuant Tyto. Dot plots show frequencies of Spike⁺IgG⁺ B cells prior to sorting in the input chamber, in the positive and negative collection chamber. Flow cytometry analysis was performed on the MACSQuant Analyzer 10.

To test the functionality of the sorted cells, B cell expansion as well as the secretion of anti-spike antibodies was examined. Sorted B cells expanded ~20 times over 14 days of culture and indicated good viabilities using the B Cell Expansion Kit. However, the magnitude to polyclonal stimulation may vary depending on the activation status of the donor's immune system, which affects the activation status and, in particular, the cell number of the target B cells.

During the analysis of the cell culture supernatants for spike-specific antibodies only the sorted SARS-CoV-2-specific B cells (Spike$^+$) secreted spike-specific antibodies further confirming functionality and specificity of the target cell population. Immunoglobulin concentration even increased over time underlining the high functionality and ability of the sorted B cells for long-term culture (fig. 4).

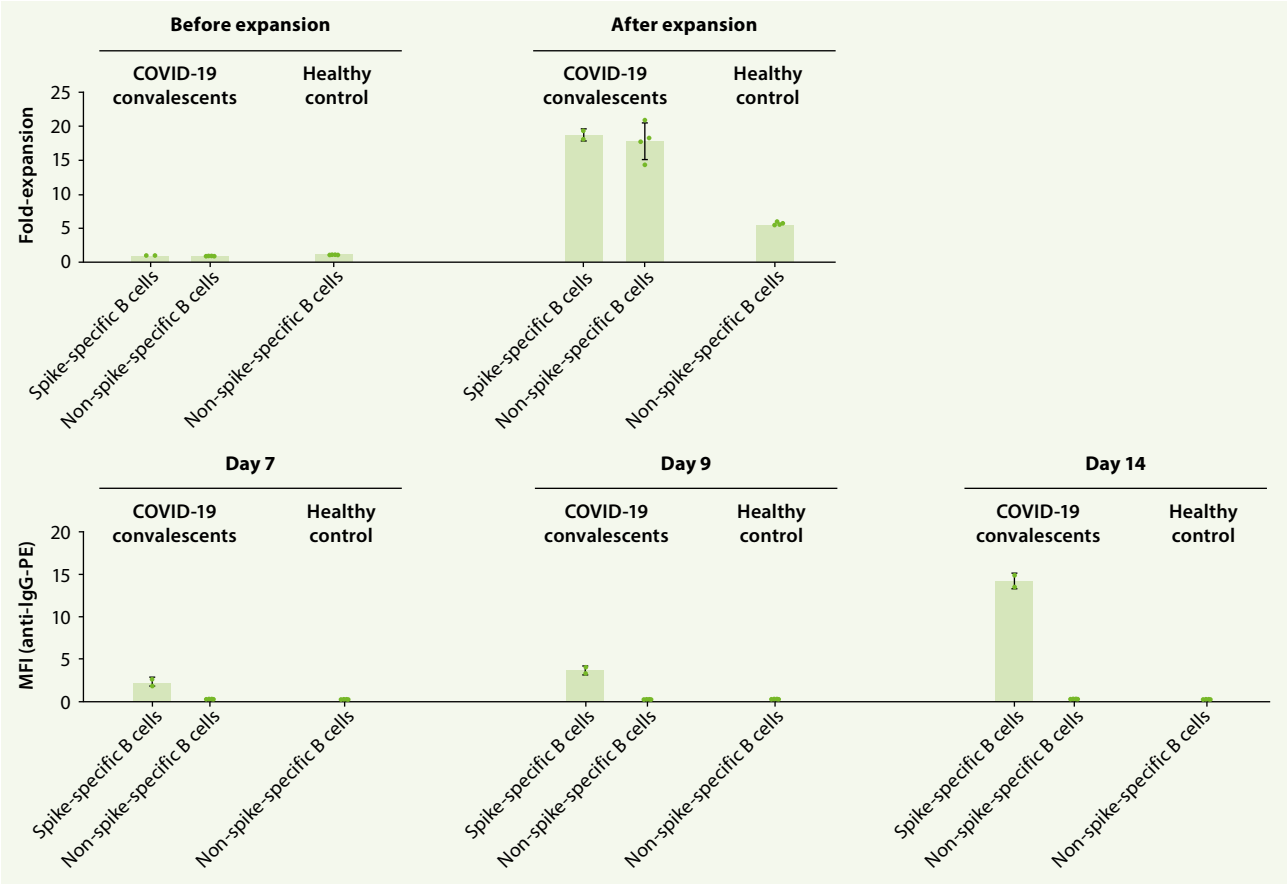

Figure 4: B cell expansion and antibody secretion after cell sorting. SARS-CoV2-specific B cells were expanded for 14 days. The fold expansion was analyzed from Spike$^+$, Spike$^-$ and healthy controls. The concentration of anti-spike-specific IgGs was measured from culture supernatants.

Conclusion

The MACSQuant Tyto offers full safety for both, the operator as well as the sample. With the closed, single-use and disposable cartridge sorting of biohazardous material like samples from SARS-CoV-2-infected patients does not pose a hurdle anymore. Numerous researchers already rely on the MACSQuant Tyto[1,2,3,4] for sorting samples from COVID-19 patients. A biosafety study also validated the MACSQuant Tyto in a BSL-3 containment setting[5].

Here, it is demonstrated that sorting of rare antigen-specific B cells with high purities is mastered by the MACSQuant Tyto. A combination of pre-enrichment using the REAlease CD19 MicroBead Kit and the MACSQuant Tyto Cartridge HS is speeding up the sorting process. Importantly, subsequent functional assays with antigen-specific B cells, a delicate cell type, are possible due to the gentle microchip-based cell sorting technique. After the sort, SARS-CoV-2-specific B cells could be expanded up to 20-fold using the B Cell Expansion Kit and a modified culture protocol. Antigen-specific B cells are rare and culturing them while preserving their physiological function is challenging since a low cell density is hampering the culture. For this reason, it is crucial to isolate this cell type gently and transfer them into culture as fast as possible. Due to the gentle sorting process, the reproducible expansion protocol and the presence of the antigen in the B cell culture, B cells expand to high rates. Variation in the expansion rates are expected according to donor-specific differences for example the current activation state of the donor's immune system.

The culture of B cells is important for studying antibody biology and their therapeutic potential. By producing antibodies in their natural source, B cell functionality and specificity or neutralization potential of individual antibodies can be studied without bias. Supernatants from the B cell culture can be stored at –20 °C and processed when needed adding an additional layer of flexibility. Here, it was observed that spike-specific IgGs were produced only by the antigen-positive B cells demonstrating the capability of their biological function after sorting and the specificity of the procedure.

In summary, the MACSQuant Tyto facilitates multiparameter cell sorting while being gentle to the cells and protecting the operator from biohazardous samples.

References

1. Bacher, P. *et al.* (2020) Immunity 53: 1258–1271
2. Dugan, H. L. *et al.* (2021) Immunity 54: 1290–1303
3. Marongiu, L. *et al.* (2021) European Journal of Immunology 10.1002/eji.202149298
4. Roberts, L. M. *et al.* (2021) Journal of Immunology ji2100608
5. Roberts, L. M. *et al.* (2021) Applied Biosafety apb.20.0065

Product table

MACS Product	Order no.
MACSQuant Tyto instrument	130-103-931
MACSQuant Tyto Cartridge HS	130-121-549
MACSQuant Tyto Running buffer	130-107-206
SARS-CoV-2 Spike B Cell Analysis Kit, human	130-128-022
REAlease CD19 MicroBead Kit, human	130-117-034
B Cell Expansion Kit, human	130-106-196
MACSQuant Analyzer 10	130-096-343

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