



Miltenyi Biotec



MACSQuant  
Tyto

# Mitigate the hazards of isolating live cells and microorganisms from high-risk samples

## MACSQuant® Tyto® Family: effortless biosafety in cell sorting

### Introduction

Sorting hazardous materials is a precarious and pricey pursuit. This is especially true in biosafety-level (BSL) labs: handling high-risk microorganisms requires time-consuming sterilization protocols, specialized biosafety infrastructure, and careful management of hazardous waste, all of which slow down workflows and raise operational costs.

The MACSQuant Tyto Family of benchtop cell sorters eliminates these hurdles with a fully closed cartridge system. Here, we show that the MACSQuant Tyto Cell Sorter enables gentle, high-purity sorting of high-risk samples without risking cross-contamination. By combining safety, simplicity, and performance, the MACSQuant Tyto Family empowers both basic and clinical research laboratories to confidently handle infectious materials while saving time, reducing costs, and protecting personnel, samples, and equipment.

### Why conventional droplet sorters put biosafety at risk

When working with high-risk material, conventional droplet-based sorters introduce serious biosafety concerns:

- **Aerosol generation:** the high-pressure sorting generates aerosols, risking operator and lab safety when sorting infectious samples.
- **Cross-contamination:** the open fluidic systems increase the risk of cross-contamination.
- **Time-consuming decontamination:** eliminating contamination in droplet sorters requires extensive and repeated sterilization procedures and cleaning routines.
- **Infrastructure demands:** to mitigate risks, labs rely on custom biosafety cabinets and complex containment systems, which are expensive and require significant lab space.

Taken together, these challenges make droplet sorters a precarious choice when working with high-risk samples, underscoring the urgent need for safer, more reliable alternatives.

### Biosafety guidelines for sorting hazardous samples

As flow cytometry sorting became increasingly common in both basic and clinical research, concerns grew over the handling of biological specimens that could contain known or unknown infectious agents.

Recognizing these hazards, the International Society for the Advancement of Cytometry (ISAC) first issued biosafety guidelines in 1997, establishing global standards for protecting both personnel and the lab environment. A decade later, in 2007, the guidelines were revised to reflect advances in flow cytometry and the growing use of unfixed, potentially infectious samples. The most recent update in 2014 further strengthened these standards<sup>1</sup>, providing recommendations for:

- laboratory design to accommodate cell sorting experiments
- laboratory- and instrument-specific standard operating procedures (SOPs)
- personal protective equipment (PPE)
- validation of aerosol containment by regular testing and risk assessment

Today, the ISAC guidelines are regarded worldwide as the benchmark for safe cell sorting practices.

### Validating aerosol safety in cell sorting

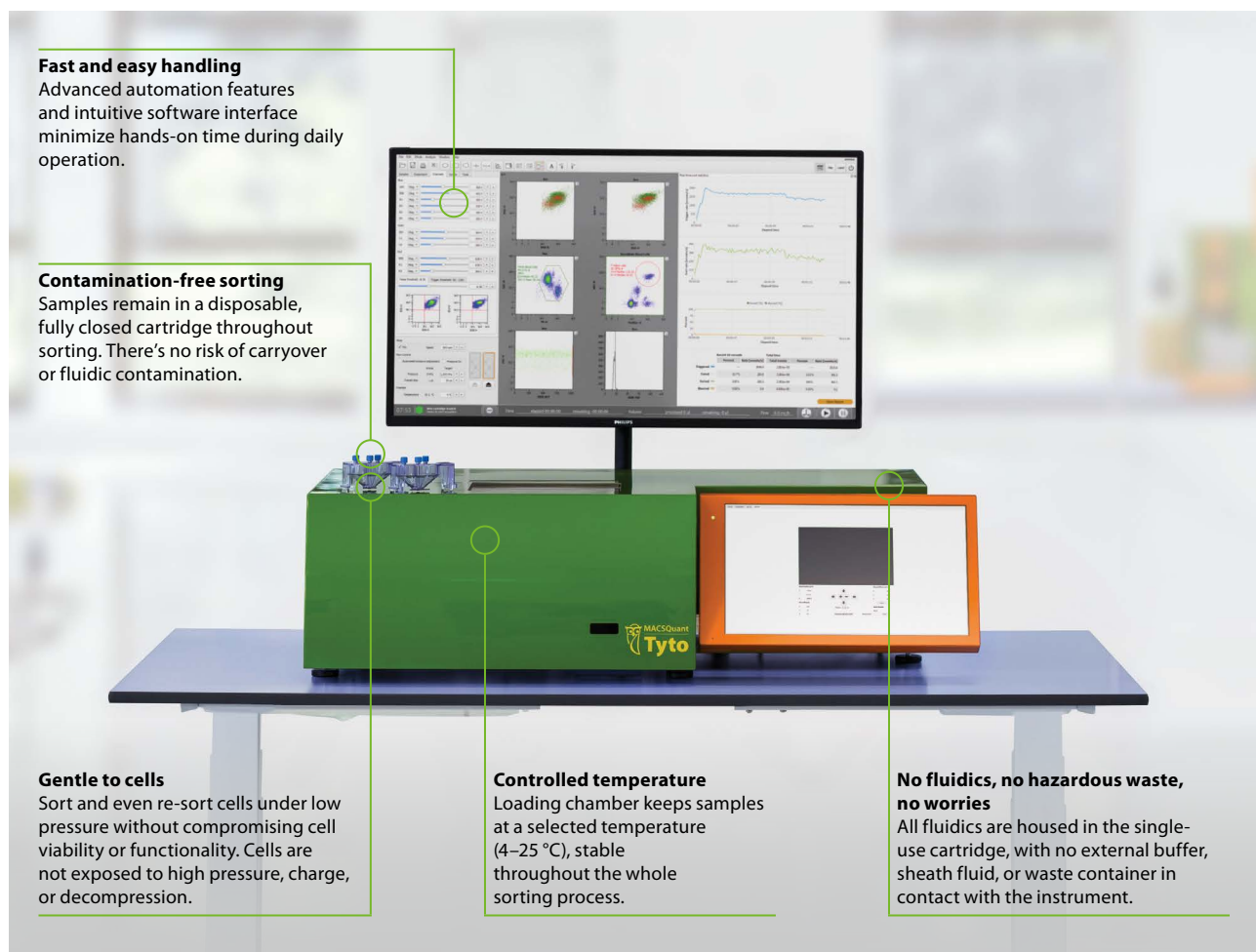
Traditionally, aerosol containment in flow cytometers and sorters has been evaluated with the Glo-Germ® assay<sup>2</sup>. In this method, a suspension of fluorescent particles is run through the instrument. To test aerosol formation, an Aerotech impactor positioned near the nozzle collects any escaping aerosols onto a glass slide, which is then examined under a fluorescence microscope. This method has several drawbacks, such as:

- the Aerotech impactor requires rigorous cleaning between measurements
- scanning the large collection slide under a fluorescent microscope is laborious and time-consuming
- the Glo-Germ particles vary in size, resulting in inconsistent flow rates

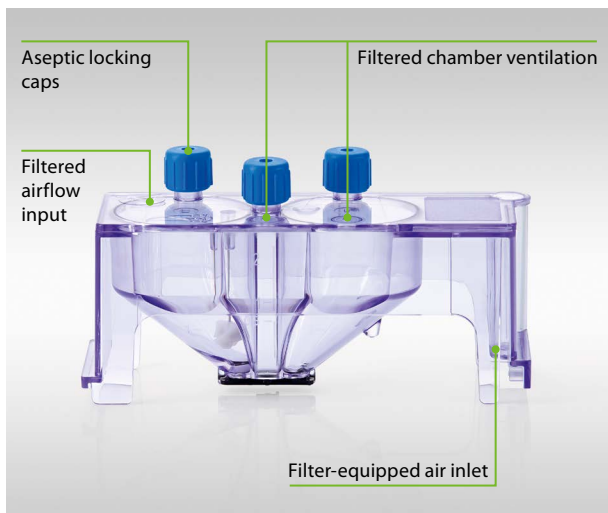
To overcome these limitations, researchers developed a more efficient approach using Dragon Green beads, uniform in size and fluorescence, and a Cyclax-d impactor that collects particles onto a narrowly defined area, improving detection sensitivity and reproducibility compared to standard Glo-Germ procedures<sup>3</sup>.

### MACSQuant Tyto Family of cell sorters

The MACSQuant Tyto Family combines advanced microfluidics and a closed cartridge system to deliver high-speed multiparameter cell sorting (fig. 1).



**Figure 1:** The MACSQuant Tyto Family of cell sorters with patented microchip-based technology opens endless possibilities in basic and clinical research with gentle cell sorting in the safety of a closed cartridge. The three-laser MACSQuant Tyto Cell Sorter is shown.



**Figure 2:** In the closed MACSQuant Tyto Cartridge system, each chamber is only accessible through aseptic Luer lock-sealed inlets. All air inlet and outlet ports are equipped with 0.1 µm hydrophobic multilayer air filters, for sterility and aerosol containment.

The sorting process takes place entirely within the disposable MACSQuant Tyto Cartridge, where samples remain sealed and sterile, never in contact with the instrument (fig. 2). The patented microchip technology and integrated temperature control mitigate sorter-induced cellular stress, enabling gentle cell sorting that preserves cell viability and functionality (fig. 3).

Using the Dragon Green beads assay, researchers validated the MACSQuant Tyto Cell Sorter under BSL-3 conditions<sup>4</sup>. Due to the fully closed cartridge system, no aerosol formation was detected, even under stringent conditions designed to stimulate a containment breach. Here, we build on these results, confirming that the benchtop MACSQuant Tyto Cell Sorters can be safely operated in high-level biosafety labs, offering a safe alternative to droplet sorters.

## Methods

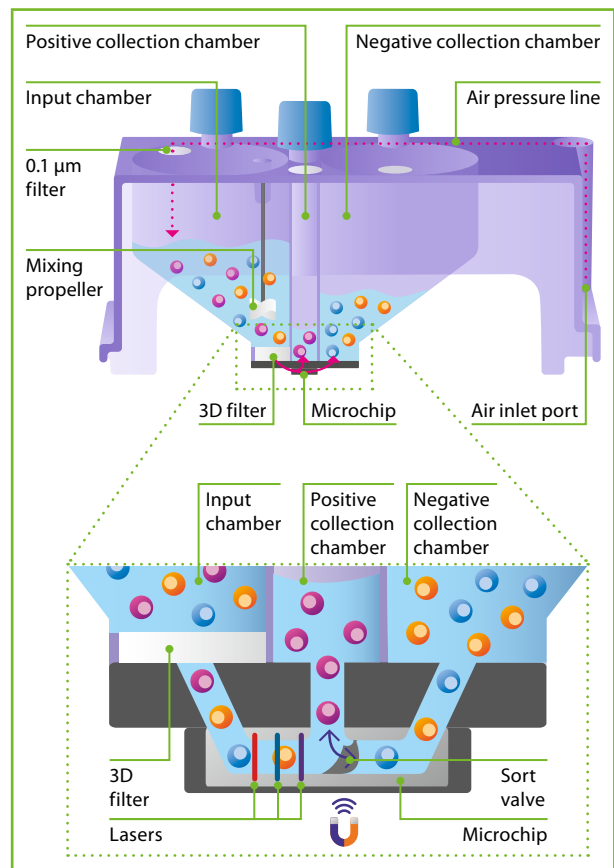
### Testing aerosol containment with fluorescent particles

Aerosol containment of the MACSQuant Tyto Cartridge was evaluated by sorting a suspension of Fluoresbrite® YG Microspheres of different sizes (1 µm, 2 µm, and 10 µm) inside the MACSQuant Tyto Cell Sorter. During the process, a Cyclax-d impactor was positioned above the air ventilation port of the negative collection chamber to capture any particles that might pass through the integrated 0.1 µm filter (fig. 4).

After sorting, the lid of the negative collection chamber was removed, and the air filter was excised for further inspection. To increase the likelihood of aerosol release, the microsphere suspension was run at 300 mbar, twice the standard operating pressure. All samples, including the filter and coverslips from the impactor, were analyzed by fluorescence microscopy.

### Cartridge integrity testing

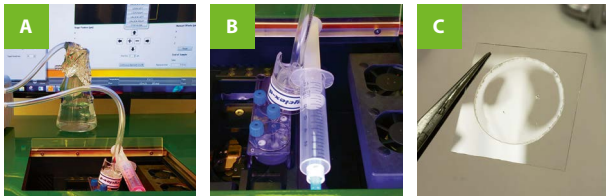
To simulate mishandling, 5 cartridges loaded with a 5 mL suspension of Fluoresbrite YG Microspheres of variable sizes (1 µm, 2 µm, and 10 µm) were dropped from a 2-meter height onto hard laminate flooring. Cartridges were examined under a stereoscope for structural damage and tested for aerosol release using the Cyclax-d impactor.



**Figure 3: The sorting mechanism of the MACSQuant Tyto Family.** Upon inserting the cartridge into the instrument, air from the instrument is directed into the cartridge through a 0.1 µm filter and traverses the air pressure line towards the input chamber. The air flow propels the cells through a 3D-filter system, guiding them into the microchannel and toward the microchip. Inside the microchip, cells are interrogated by laser beams. Fluorescent and scattered light signatures are used to identify cells. Non-target cells (orange, blue) bypass the closed valve into the negative collection chamber. Once a target cell is identified (pink), the sorting valve inside the microchip opens, directing the target cell into the sorting chamber. The valve then returns to its original position and is ready for the next sort. The infographic illustrates the three-laser detection system of the MACSQuant Tyto Cell Sorter.

### Sorting of high-risk samples

Samples containing GFP-positive *Plasmodium berghei* sporozoites ( $2.8 \times 10^5$  cells/mL) and samples containing GFP-expressing *Escherichia coli* were sorted using the MACSQuant Tyto Cartridge on the MACSQuant Tyto Cell Sorter. Original (input) and sorted (positive) fractions were analyzed by flow cytometry on the MACSQuant Analyzer to determine purity. Microscopy was performed with both brightfield and fluorescence to assess sporozoite post-sort motility. Bioburden testing was performed to detect potential bacterial carryover between sorted samples.



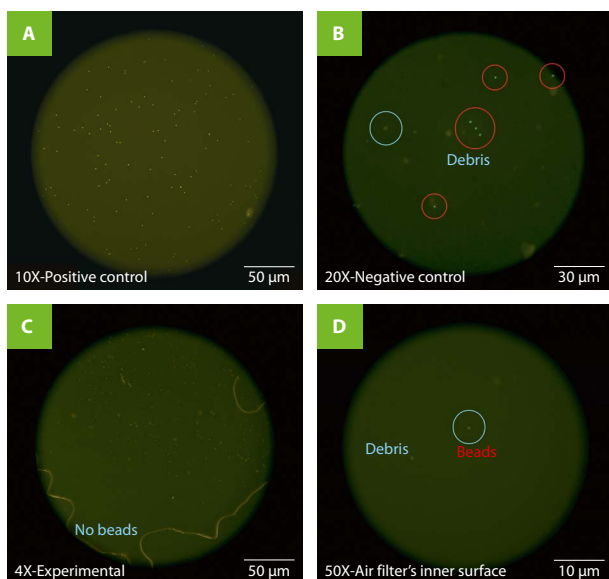
**Figure 4: Testing aerosol containment in the MACSQuant Tyto Cartridge during sorting.** The loading chamber on the MACSQuant Tyto Cell Sorter was kept open for aerosol testing during the sort of fluorescent microspheres (A). The Cyclax-d impactor was positioned above the negative collection chamber (B). The impactor collected aerosols into a coverslip for posterior analysis (C).

## Results

### The MACSQuant Tyto Cartridge prevents aerosol formation and release

To assess potential aerosol escape, a suspension of fluorescent beads of different sizes was sorted through the MACSQuant Tyto Cartridge. Assay sensitivity was confirmed using an ultrasonic atomizer, which generated abundant aerosols from a microsphere suspension, captured by the impactor (fig. 5A). As a negative control, sorting a cartridge loaded only with PBS produced no fluorescent signal in the impactor sample (fig. 5B). When fluorescent beads were sorted, no aerosols were detected by the impactor outside the cartridge, in any experiment (fig. 5C). Only a very small number of 1  $\mu\text{m}$  particles were observed on the inner surface of the cartridge filter (fig. 5D), demonstrating the filter's ability to capture rare particle events and demonstrating the absence of meaningful aerosol formation within the cartridge itself.

These findings suggest that even under high pressure, the closed MACSQuant Tyto Cartridge prevents significant aerosol generation. In the rare event of generating aerosols, the integrated air filters effectively trap particles  $\geq 1 \mu\text{m}$ , the size of most bacteria, yeast, and parasites, indicating that the closed cartridge system can safely contain these microorganisms.



**Figure 5: The air filters on the MACSQuant Tyto Cartridge safely contain rare 1  $\mu\text{m}$  aerosol particles.** Aerosolization using an ultrasonic atomizer was used as positive control (A). Sorting of a PBS-loaded cartridge was used as negative control (B). Fluorescent beads were never detected in the impactor samples (C). A small number of 1  $\mu\text{m}$  microspheres were detected on the inner surface of the cartridge filter (D).

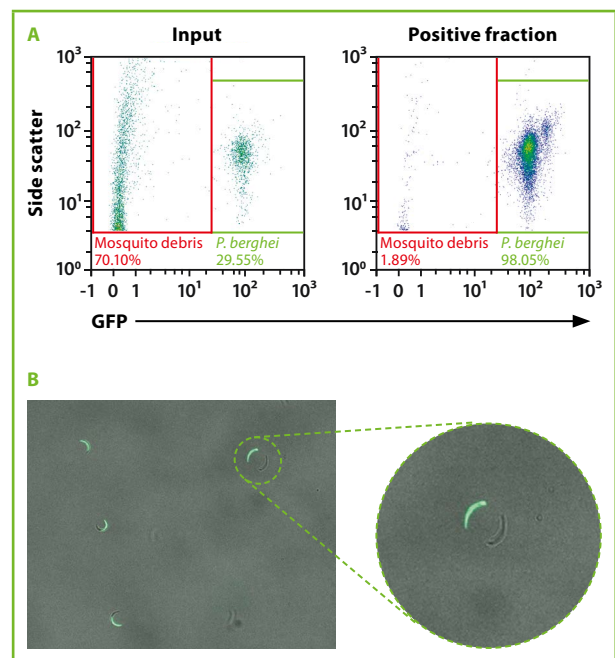
### Cartridge integrity is maintained after dropping

To assess robustness, bead-loaded MACSQuant Tyto Cartridges were dropped from a height of two meters. All five cartridges remained fully intact, showing only minor cosmetic scratches on the polycarbonate body. The cartridge's structural design, extending beyond the chip surface, protected the sensitive internal components from direct impact. No leaks were observed, and the adhesive seal between the interposer and body remained secure. No microspheres were detected in the impactor during follow-up sorting runs, confirming the absence of aerosol release or leakage after impact.

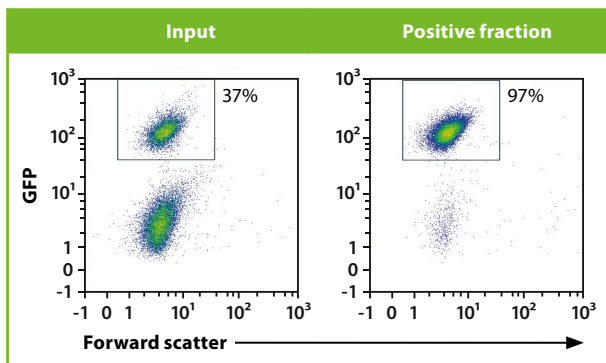
### Gentle and safe sorting of delicate *Plasmodium* sporozoites

Malaria, caused by *Plasmodium* parasites, begins when sporozoites from an infected mosquito enter the human body. The morphology of *Plasmodium* varies by developmental stage and species, appearing as elongated, spherical, ring-, or crescent-shaped forms that range from 1 to 20  $\mu\text{m}$  in diameter<sup>5</sup>. Isolating the sporozoite stage of *Plasmodium* using conventional droplet sorters is challenging. High sorting pressures, electrical charges, and rapid decompression can damage the cells, while preventing cross-contamination between samples requires labor-intensive and time-consuming sterilization procedures.

Using the MACSQuant Tyto Cell Sorter and the closed MACSQuant Tyto Cartridge, GFP-positive *Plasmodium berghei* were enriched from a target cell fraction of 30% to 98% purity in the positive fraction (fig. 6A). Time-lapse microscopy, capturing both fluorescence and brightfield images, confirmed that sporozoites remained motile after sorting (fig. 6B), demonstrating that the MACSQuant Tyto Family preserves viability and functionality of even the most delicate cells. The closed cartridge system delivers gentle sorting while reducing biosafety risks, eliminating the need for extensive sterilization.



**Figure 6: Gentle and safe sorting of delicate *Plasmodium* sporozoites.** The MACSQuant Tyto Cell Sorter enabled high-purity sorting of GFP-positive *P. berghei* under gentle conditions, increasing purity from 30% in the input fraction to 98% in the sorted fraction (A). Time-lapse microscopy confirmed sporozoite movement immediately after sorting, indicating preserved viability (B).



**Figure 7:** The MACSQuant Tyto Cell Sorter enabled high-purity sorting of GFP-positive *E. coli*, enriching the population from a target frequency of 37% in the input fraction to 97% purity in the positive fraction.

### High-purity sorting of bacteria without risk of cross-contamination

Sorting hazardous bacterial samples is often limited by the need for time-consuming and labor-intensive sterilization protocols. *E. coli*, a gram-negative, rod-shaped bacterium (1–2 µm long, with about 0.5 µm radius)<sup>6</sup>, serves as a model organism for evaluating safe sorting workflows.

Using the MACSQuant Tyto Cell Sorter, GFP-expressing *E. coli* were enriched from a target frequency of 37% in the input sample to 97% purity in the positive fraction (fig. 7). Immediately after sorting, a new cartridge loaded with fresh media was inserted and run for 2 hours. This second cartridge was then incubated under standard *E. coli* culture conditions. Follow-up bioburden testing after 14 days showed zero cross-contamination in the second cartridge (<1 CFU/mL, data not shown).

These results demonstrate that the closed MACSQuant Tyto Cartridge enables high-purity bacterial sorting while eliminating the need for complete instrument sterilization between runs.

## Conclusions

Working with hazardous or infectious material in conventional droplet sorters comes with well-known risks: aerosol generation, cross-contamination, and the need for extensive sterilization and biosafety infrastructure. In this study, we demonstrated how the MACSQuant Tyto Family overcomes these limitations by combining safety, robustness, and gentle cell handling in a fully closed cartridge system. From bacteria to parasites, the system consistently delivers high-purity sorting without compromising biosafety or cell integrity:

### Aerosol-free safety

The fully closed cartridges prevent aerosol formation, protecting personnel and reducing the need for costly biosafety infrastructure.

### Leak-proof robustness

The MACSQuant Tyto Cartridges are designed to withstand accidental impacts without compromising its structural integrity or containment.

### Gentle on delicate samples

The microchip-based sorting technology enables gentle cell sorting that preserves cell viability and functionality.

### Contamination-free sorting

Consecutive bacterial sorts showed no carryover, eliminating the need for time-consuming sterilization procedures between runs.

By combining safety, simplicity, and uncompromising performance, the MACSQuant Tyto Family stands as the most reliable and future-ready alternative to droplet sorters. It empowers laboratories to safely expand their sorting capabilities to high-risk or delicate samples while saving time, cutting costs, and enabling the highest level of biosafety.

VISIT 



See more data: safe sorting of high-risk samples with the MACSQuant Tyto Family.

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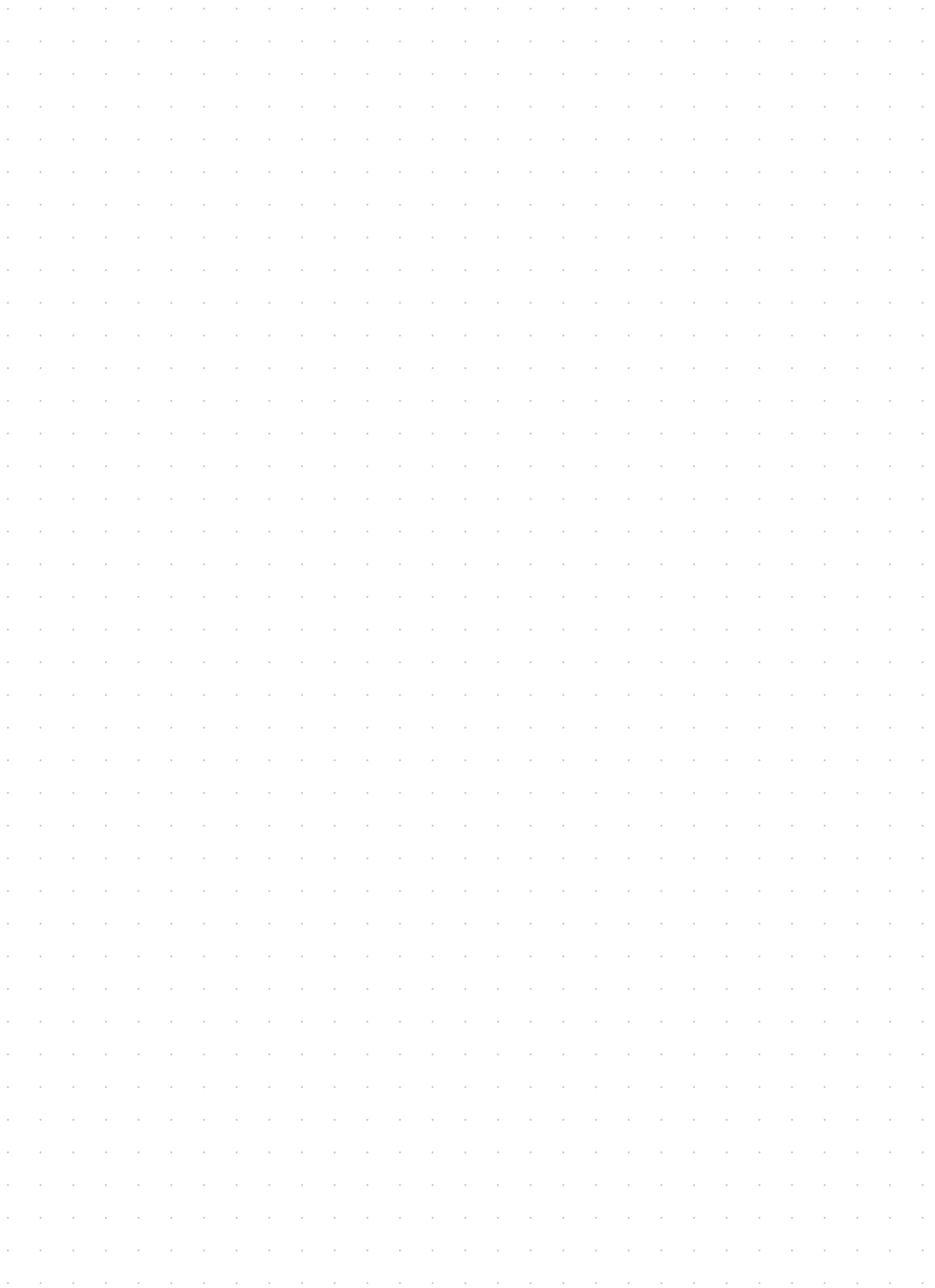
Explore the MACSQuant Tyto Family and unlock new possibilities in cell sorting today!

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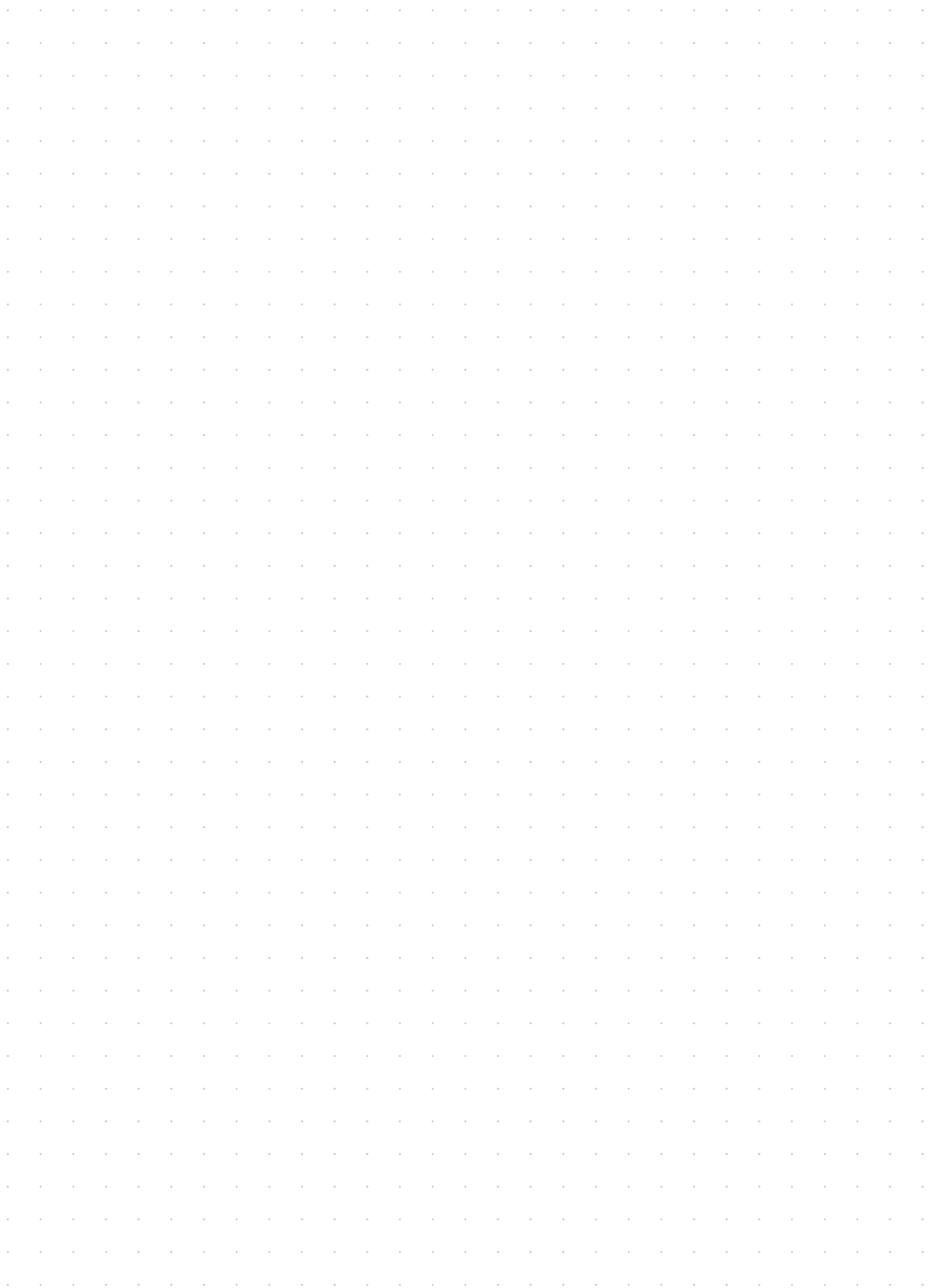
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## Notes



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**Miltenyi Biotec**

**Miltenyi Biotec B.V. & Co. KG** | Phone +49 2204 8306-0 | Fax +49 2204 85197 | [macsde@miltenyi.com](mailto:macsde@miltenyi.com) | [www.miltenyibiotec.com](http://www.miltenyibiotec.com)

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