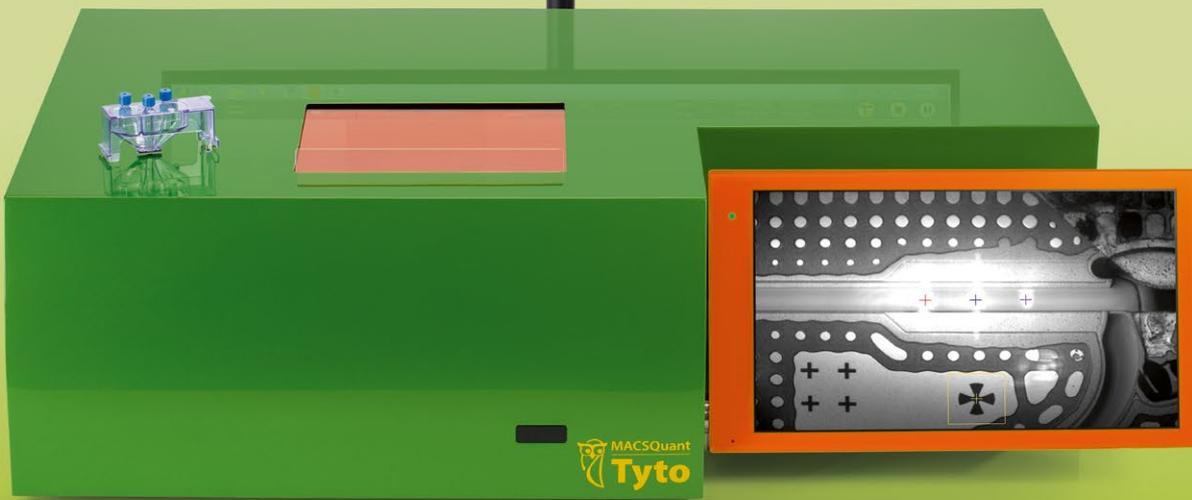
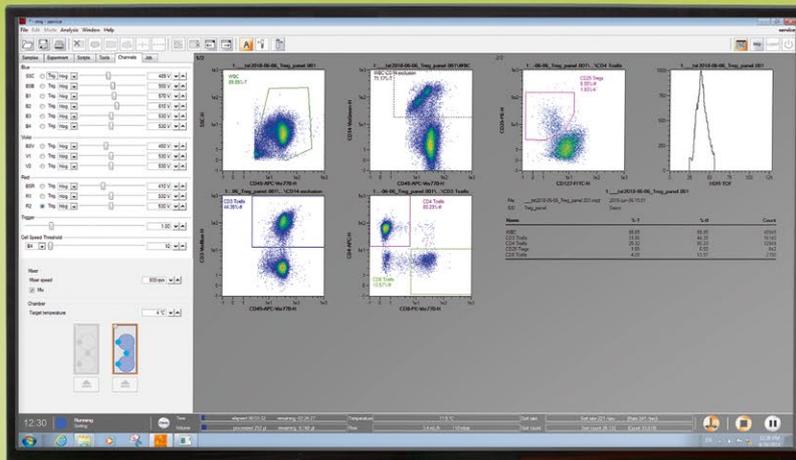




Miltenyi Biotec

# MACSQuant<sup>®</sup> Tyto<sup>®</sup> Cell Sorter

The revolution in cell sorting has begun



**JOIN THE  
FLOW  
REVOLUTION**

# A leap forward in cell sorting

The MACSQuant Tyto is revolutionizing cell sorting. Our patented microchip-based technology opens new possibilities in basic research and medical applications with high-speed, multi-parameter flow sorting in the safety of a fully enclosed cartridge.

## Full sterility

Samples are kept contamination-free within the disposable, fully closed MACSQuant Tyto Cartridge. No risk of carry-over or contamination of fluidics.

## Fast and easy

No drop delay or laser alignment needed. Simply insert the cartridge, gate on cells, and sort.

## Complete safety

The fully closed cartridge prevents aerosol and droplet formation, providing a safe environment for operator and sample.

## Gentle to cells

Sort and even re-sort cells under low pressure without compromising cell viability or functionality. Due to the revolutionary microchip-based sorting principle, cells are no longer exposed to charge, decompression or high pressure.





# The revolutionary sorting principle

The MACSQuant Tyto is a next generation, benchtop cell sorter equipped with 3 lasers, which allows for 10-parameter cell sorting. A unique feature of the instrument is the fact that the actual sorting process takes place exclusively within the MACSQuant Tyto Cartridge. The cartridge provides a single-use, disposable and fully closed system, eliminating the risk of sample contamination and carry-over.

No fluidics in the systems, sorting happens exclusively within the MACSQuant Tyto Cartridge

## The MACSQuant Tyto Cartridge

The MACSQuant Tyto Cartridge consists of three compartments that can be accessed from above: the input chamber, the positive, and negative collection chambers (fig. 1). Fluorescently labeled cells are loaded into the input chamber, which contains an adjustable propellor to keep cells in suspension. The cartridge houses the actual heart of the sorting system: the microchip (fig. 2).

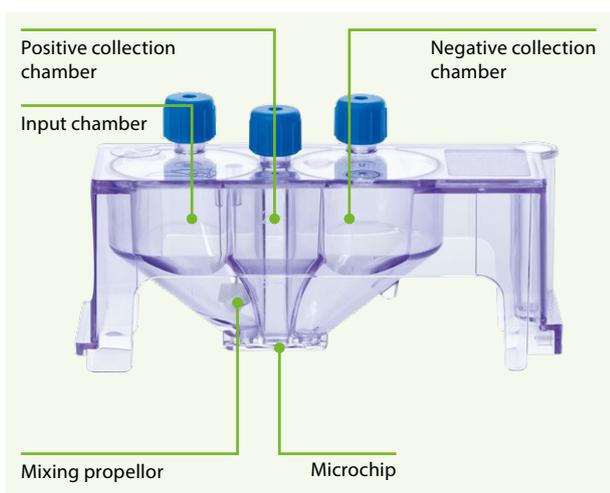


Figure 1: The MACSQuant Tyto Cartridge.

## Microchip technology

Located at the bottom of the MACSQuant Tyto Cartridge (fig. 2), the microchip enables high-speed, fluorescence-based cell sorting with the world's fastest mechanical sort valve (30,000 valve actuations per second). The valve redirects cells into either the positive or negative collection chamber, without the formation of droplets. Unlike during conventional droplet sorting procedures, cells do not experience high pressure, they are not decompressed, and no charge is applied, resulting in great viability and functionality of cells.

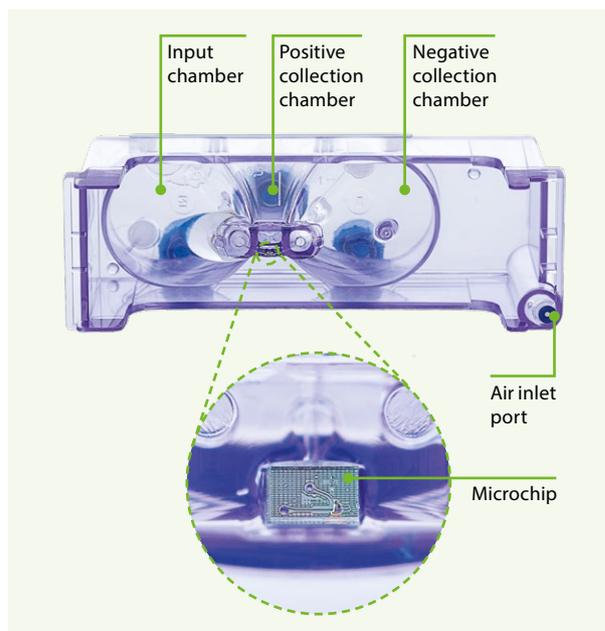
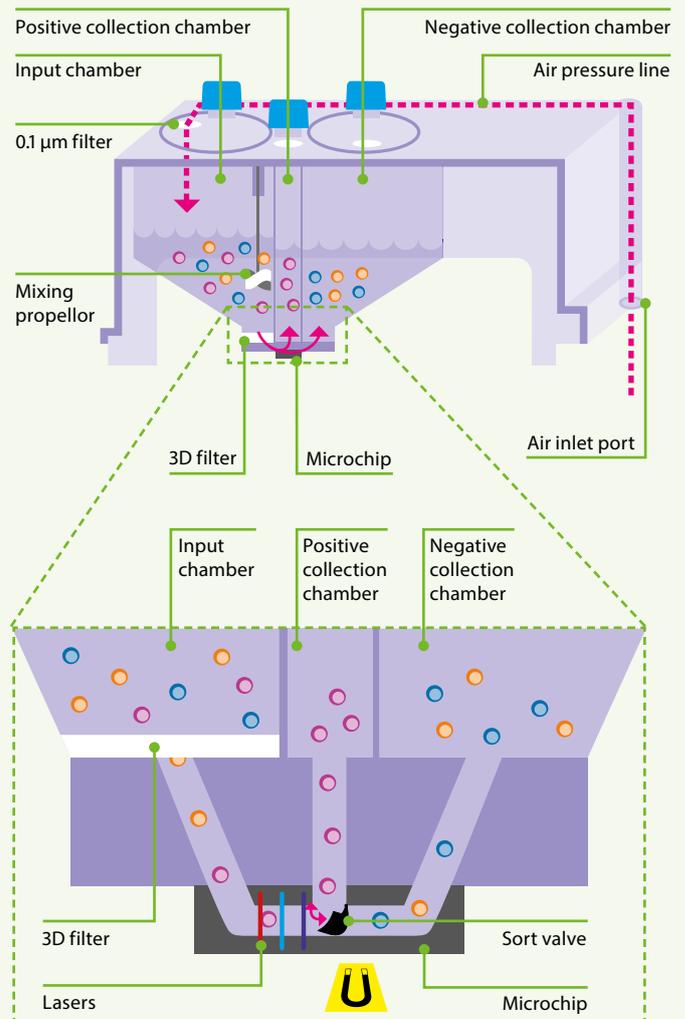


Figure 2: Bottom view of the MACSQuant Tyto Cartridge with zoom-in on microchip.

# Unique microchip-based cell sorting step by step

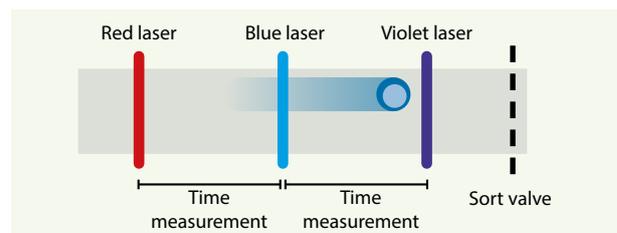
- 1** Filtered air coming from the instrument enters through a 0.1  $\mu\text{m}$  filter within the air inlet port at the bottom of the cartridge and flows through the air pressure line towards the input chamber. The air enters the input chamber through another 0.1  $\mu\text{m}$  filter, driving the cells through a microchannel into the microchip at very low pressure (<3 PSI). Before entering the microchannel, potential cell aggregates are held back by a filter system guaranteeing a smooth sorting process.
- 2** Within the microchip, cells are interrogated by three lasers. Based on their fluorescent and scatter light signatures, target cells are redirected by a sort valve located within the microchannel (see magnification). The default destination of cells is the negative collection chamber.
- 3** Once a target cell is identified, a magnetic field is applied to the microchip. This triggers the sorting valve to open and therefore redirects the target cell into the positive collection chamber. After a positive cell has passed through, a silicon spring returns the valve into its original position.



## Automated valve timing

For optimal sort performance, precise valve timing is determined for every individual cell.

How does this work? When a cell passes two subsequent lasers, the cell speed of this particular cell is measured by determining the travel time between those lasers. Based on the measured speed, the exact opening timepoint and opening duration of the sort valve are adjusted (fig. 3).



**Figure 3: Individual cell speed measurements allow for precise valve timing.**



Reliable



Closed system



Translational



# Full sterility

The MACSQuant Tyto Cartridge provides a completely closed and sterile system, in which samples are fully protected from environmental contamination. In contrast to conventional droplet sorters, the MACSQuant Tyto Cell Sorter does not use droplets to separate cells, eliminating biohazardous aerosol formation.

The air used to pressurize the input chamber and control the sorting flow within the cartridge is pre-filtered. In addition, pressure outlets on the positive and negative chambers are sealed with 0.1  $\mu\text{m}$  filters that allow for sterile ventilation of your sample.

All cartridges undergo a validated and strictly monitored sterilization process that uses ethylene oxide to guarantee full sterility of each cartridge lot.



# Fast and easy

## Simple loading, automated sort setup and operator-free sorting

The MACSQuant Tyto Cell Sorter can easily be operated by lab professionals without intensive training. Simply load your sample under a sterile hood, and scan the cartridge using the barcode scanner, which automatically detects cartridge type and lot. Loading of the cartridge into the instrument proceeds in an intuitive “plug-and-play” fashion.

Flow control, laser alignment, speed detection and valve timing are fully automated features on the MACSQuant Tyto Cell Sorter, thereby eliminating the need for specialized technical expertise during daily operation. Since the actual sorting process completely takes place within the cartridge, there is no risk of losing precious sample material. Both, positive and negative sort fractions can easily be retrieved under a sterile hood.

Conveniently, sorting on the MACSQuant Tyto Cell Sorter is entirely hands-off, saving time, resources, and thus money.



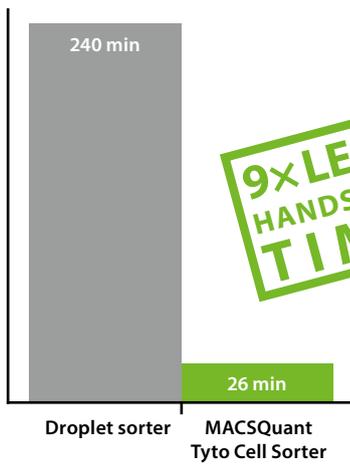
Droplet Sorter	
Step	Time
<ul style="list-style-type: none"> <li>• Device/software startup</li> <li>• Fluidic startup and stabilization</li> <li>• Nozzle selection and setting sheath pressure</li> <li>• Defining sort settings</li> <li>• Adjust drop drive amplitude and frequency</li> <li>• Focus side streams</li> <li>• Drop delay calculation</li> <li>• Turn on stream monitoring</li> <li>• Aim sort streams</li> <li>• Instrument QC</li> </ul>	00:30 – 01:00 h
<ul style="list-style-type: none"> <li>• Experiment setup</li> </ul>	00:05 h
<ul style="list-style-type: none"> <li>• Sorting</li> </ul>	02:30 h – operator required
<ul style="list-style-type: none"> <li>• Cleaning</li> </ul>	00:05 h – cleaning between users (without exchanging fluidics)
<ul style="list-style-type: none"> <li>• Shutdown</li> </ul>	00:20 h – complete shutdown
<b>Overall operator time committed to instrument</b>	<b>≥03:30 – 04:00 h</b>

MACSQuant Tyto Cell Sorter	
Step	Time
<ul style="list-style-type: none"> <li>• Device/software startup</li> <li>• Defining sort settings</li> <li>• Instrument QC</li> </ul>	00:15 h
<ul style="list-style-type: none"> <li>• Experiment setup</li> <li>• Cartridge filling and alignment</li> </ul>	00:10 h
<ul style="list-style-type: none"> <li>• Sorting</li> </ul>	02:30 h – operator-free
<ul style="list-style-type: none"> <li>• Cleaning</li> </ul>	Not required
<ul style="list-style-type: none"> <li>• Shutdown</li> </ul>	≤00:01 h
<b>Overall operator time committed to instrument</b>	<b>≥00:26 h</b>

**Table 1:** Comparison of overall hands-on time between the MACSQuant Tyto Cell Sorter and a state-of-the-art droplet-based sorting device for a typical sort of 2.5 h.



Only 26 minutes hands-on time



Operator-free sorting



# Complete safety

## Sort cells safely and contamination-free

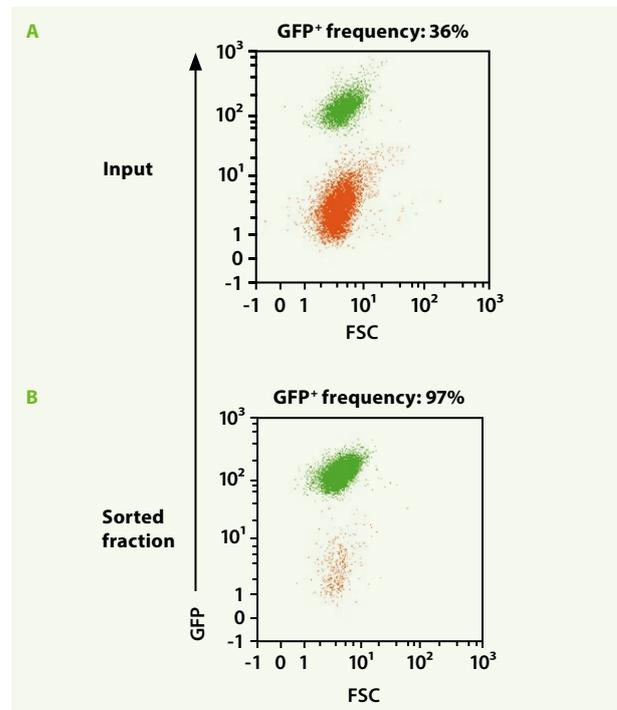
The single-use disposable MACSQuant Tyto Cartridge completely eliminates the risk of external contamination and sample-to-sample carry-over. Therefore, you can be confident that your sorted cells are ready for use in sensitive downstream applications such as cell culture.

Additionally, the closed character of the cartridge eliminates the formation of biohazardous aerosols or droplets, resulting in maximum operator safety.

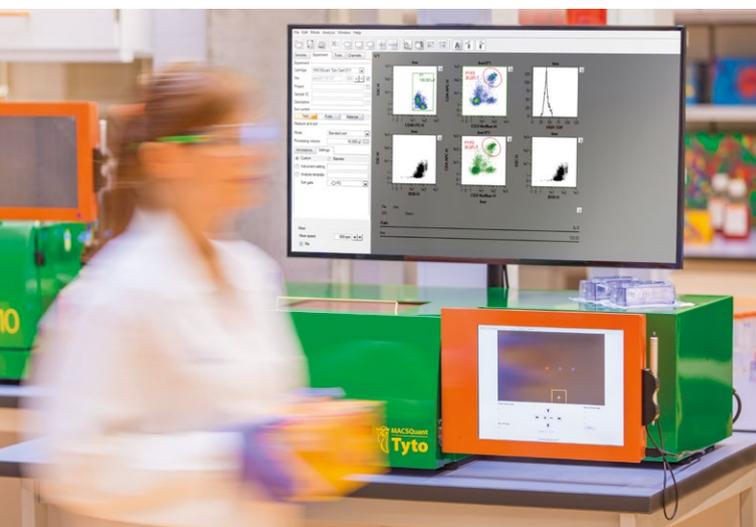
## Sorting of high-risk material

Many operators have to take special precautions when faced with the challenging task of sorting risk material. Bacterial or yeast sorts always pose the danger of sample-to-sample carry-over as well as contamination of the instrument. In addition, conventional droplet systems are typically housed in space-consuming safety cabinets in order to guarantee operator safety.

In contrast, the safe design of the MACSQuant Tyto Cartridge excludes these hurdles. High-risk material such as bacteria or yeast can be sorted efficiently (fig. 4), without the risk of persistent contamination of your cell sorter. Additionally, a complex cleaning routine of the instrument between samples or special housing precautions are not necessary, saving time and money.



**Figure 4: Sorting of bacteria.** A mixture of wildtype (orange) and GFP-expressing (green) *E. coli* was sorted on the MACSQuant Tyto Cell Sorter. The different fractions were analyzed on the MACSQuant Analyzer 10 before (input) and after the sort. (A) shows the original fraction with 37 % GFP<sup>+</sup> *E. coli* before sorting, and (B) the enriched positive fraction with 97 % GFP<sup>+</sup> *E. coli* after the sort.



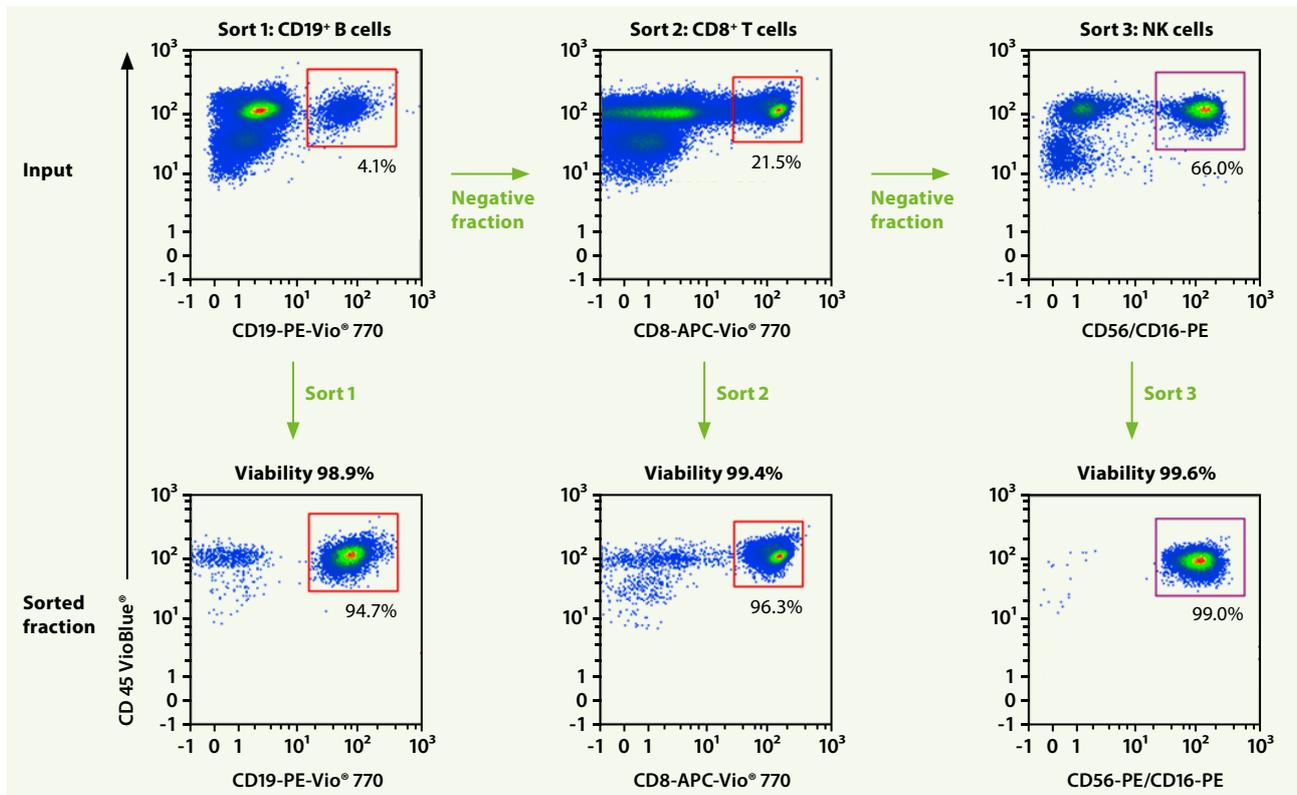




# Gentle to cells

Unlike on conventional droplet sorters, cells sorted by the MACSQuant Tyto do not experience high pressure or charge, and do not get decompressed. This gentle sorting approach results in never-before-seen viability (fig. 5) and functionality of cells. Even after multiple sequential sorts, cell viability is not affected, demonstrating the gentle nature of the valve-mediated sorting mechanism.

Additionally, the MACSQuant Tyto Cell Sorter is equipped with a temperature-controlled cooling unit, stabilizing your samples at a temperature of your choice, ranging between 4–25 °C.



**Figure 5: Subsequent sorting of leukocyte populations.** In this experiment three different subpopulations were subsequently sorted out of one population of peripheral blood mononuclear cells (PBMCs), starting with CD19<sup>+</sup> B cells for the first sort. The negative fraction was used as input for a subsequent second sort, enriching CD8<sup>+</sup> cytotoxic T cells. In the third sort, the negative fraction of the second sort was used as input to sort for CD14<sup>-</sup>/CD3<sup>-</sup>/CD56<sup>+</sup>/CD16<sup>+</sup> NK cells. Sorting sessions lasted around one hour each. All fractions showed viabilities greater than 98%, even after three subsequent sorting rounds.

# The complete package for reproducible results in flow cytometry

Achieving maximum reproducibility between experiments cannot depend on the instrument alone. In order to achieve consistent results, Miltenyi Biotec offers a complete flow cytometry solution including a broad range of reagents. We help you make sure that variations in your experiment are due to your sample and not due to unreliable antibodies or instruments.

## REAffinity™ Recombinant Antibodies – Flow cytometry is in their genes

Miltenyi Biotec has introduced a portfolio of REAffinity Recombinant Antibodies that provide superior lot-to-lot consistency and purity compared to mouse or rat monoclonal and polyclonal antibodies. Our recombinant technology also diminishes the need for FcR blocking and allows for analyses with only one single isotype control, generating high quality data with no more background signal while saving efforts when setting up experiments. For more information, visit: [www.miltenyibiotec.com/reaffinity](http://www.miltenyibiotec.com/reaffinity)

### Advantages of REAffinity Recombinant Antibodies:

- High lot-to-lot consistency
- One universal isotype
- No more background signal



## REAlase® Fluorochrome Technology – The next step of flexibility in cell sorting

REAlase Fluorochrome Technology relies on recombinantly engineered antibody fragments that are, unlike conventional antibodies, characterized by low epitope binding affinities. A novel covalent conjugation approach allows for multimerization and fluorescent labeling of these antibody fragments. By adding the REAlase Release Reagent, label-free cells can be obtained after cell sorting. For more information, visit:

[www.miltenyibiotec.com/realease-fluorochromes](http://www.miltenyibiotec.com/realease-fluorochromes)



## Customized solutions

Miltenyi Biotec's custom antibody design service enables researchers to benefit from personalized flow cytometry solutions. This service includes purified, functional-grade antibodies, single- and multicolor antibody conjugates as well as multicolor antibody cocktails. To find out more, visit:

[www.miltenyibiotec.com/customab](http://www.miltenyibiotec.com/customab)

## Support at your fingertips

Our technical support team offers around-the-clock troubleshooting (24 hours on weekdays) with realtime access to your instrument using the remote support function.

## MACSQuant® Tyto® Cell Sorter Specifications

### Optics

Laser excitation	405 nm, 100 mW   488 nm, 100 mW   638 nm, 100 mW	
Emission detectors	Channel: V1, V2 B1, B2, B3, B4 R1, R2	Filter: 450/50 nm, 525/50 nm 525/50 nm, 585/40 nm, 655–730 nm, 750 nm Long-Pass 655–730 nm, 750 nm Long-Pass
Fluorescence performance	Backscatter and side scatter for detection of size and granularity of cells 5 decade logarithmic scales, display in lin, log, or hlog scales	

### Performance

Purity	>99%
Cell flow rate	Up to 55,000 cells/s*
Sort rate	Up to 30,000 valve actuations/s*
Viability	>99% for lymphocytes
Sorting mode	Two-way sorting of positive (sorted) cells and negative (non-sorted) cells
Technical support	Built-in live support functionality for reliable support in real time
Operating pressure	<210 mbar (3 psi)
Temperature control range	4–25 °C (under standard environmental conditions)
Carry-over	No sample-to-sample carry-over due to single-use disposable cartridge

### Data management

Measurement parameters	Height measurements for all parameters
Signal processing	16-bit analog-to-digital conversion and signal processing
Operating system	Core i7, 512 GB flash disk, 8 GB DDR4 RAM and Windows® 7 64-bit
Compensation	Full 8x8 matrix compensation
Data files	.mqd (proprietary file type); .fcs (2.0, 3.0, 3.1 compatible)

### Operation details

Dimensions	965x585x280mm <sup>3</sup> : without external monitor 965x585x760mm <sup>3</sup> : with external monitor
Weight	62 kg (137 lbs): without external monitor 67 kg (148 lbs): with external monitor
Monitors	32" display and 15.6" touchscreen (internal)
Power requirements / consumption	100–240 V~, 50/60 Hz/300 W: without external monitor 100–240 V~, 50/60 Hz/500 W: with external monitor
Ports	4x USB 2.0, 6x USB 3.0, 2x Ethernet, DVI-D

### Environmental conditions

Operating temperature/humidity	18–25 °C/0–85% relative humidity, non-condensing
Emission sound pressure <70 dB(A) level at workstation	<70 dB(A)

### Cartridge details

Sterility	ETO validated sterilization method to guarantee sterility of each cartridge
A-septic filters	0.1 µm hydrophobic filters at air inlet and outlet ports
Sorting channel width	25x50 µm
Maximum loading volume (input)	100 µL–10 mL
Maximum loading cell number	5x10 <sup>7</sup> cells/mL
Cartridge alignment for laser positioning	Continuous automated cartridge alignment also during the sort
Cell retrieval	Negative sorted cells are kept in the negative collection chamber
Barcode labeling	Cartridges are labeled with a barcode for easy identification and traceability

\*dependent on input sample and Poisson statistics

Product table	Order no.
MACSQuant Tyto Cell Sorter	130-103-931
MACSQuant Tyto Cartridges	130-106-088
MACSQuant Tyto Running Buffer	130-107-206

For more details visit [www.miltenyibiotec.com/tyto](http://www.miltenyibiotec.com/tyto)



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