



Miltenyi Biotec

MACS[®] Cell Pre-Enrichment for flow sorting and analysis

Saves flow sorting time

Improves cell viability

Enhances quality of
flow cytometry data



MACS® Cell Pre-Enrichment for flow sorting

Time-saving cell processing, improved sort quality

Saving flow sorting time by MACS® Cell Pre-Enrichment

- Total sorting time reduced by up to 80%
- Target population can be sorted directly after pre-enrichment

Sorting large numbers of cells or rare cell populations can take a long time and often compromises cell viability. Pre-enrichment of these cells based on MACS MicroBead Technology solves both of these problems as it takes less than 30 min and eliminates all unwanted cells and debris. For example, magnetic separation of 5×10^8 cells prior to processing on high-speed cell sorters effectively reduces the sorting time from 7 hours to 1.4 hours. MACS MicroBeads are compatible with flow sorting and cells can be sorted and analyzed right after pre-enrichment without further treatment.

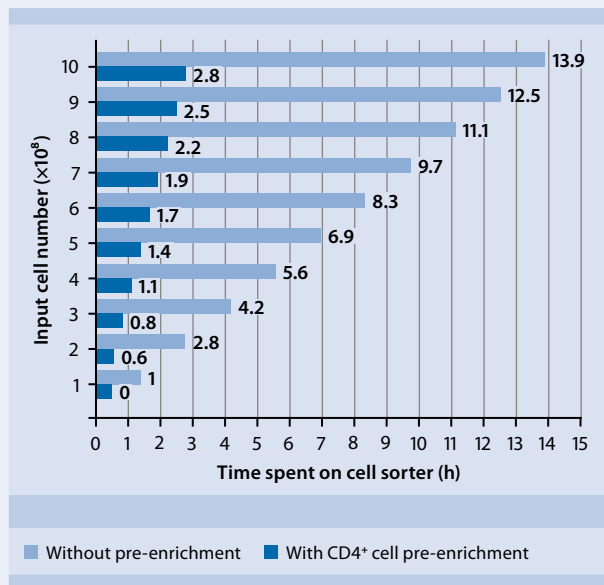


Figure 1: Time spent for sorting of CD4+ subsets with and without pre-enrichment. The bar graph demonstrates an example of total time spent on a cell sorter when sorting mouse CD4+ cell subpopulations directly (light blue bar) or pre-enriched by MACS Technology (dark blue bar) at a sorting speed of 20,000 events/s. Depending on cell numbers, time savings can be up to five-fold, from 14 h to less than 3 h when sorting 1×10^9 cells.

Improving cell viability and sort quality

- Removal of cell debris prior to cell sorting
- Reduction of sorting time increases cell viability rate

MACS Cell Pre-Enrichment saves sorting time by eliminating cell debris and non-target cells. Removing cell debris before sorting increases the efficiency and minimizes the percentage of abort events caused by non-specific influence of the debris. Using a clean sample is important for precise high-speed cell sorting, in particular for sorting rare cell populations (<1%) where high abort event rates often lead to a low cell yield. Shorter total sorting time also increases the percentage of viable cells since the cells spend less time in the collection tubes before and after processing.

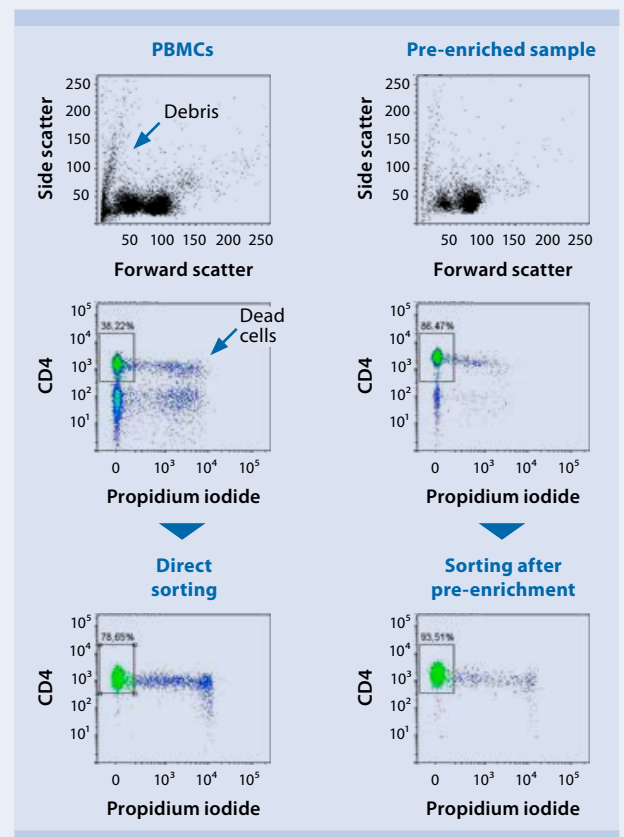


Figure 2: Higher cell purity and viability with pre-enrichment. CD4+ subsets were sorted from human PBMCs with and without pre-enrichment. Due to gentle pre-enrichment by the CD4+ T Cell Isolation Kit and shorter subsequent sorting, the number of viable cells is much higher (93.5%) than in the sample sorted without pre-enrichment (78.6%).

MACS® Cell Pre-Enrichment for flow sorting

Selected products

Selected cell separation reagents for pre-enrichment of human cells

Pre-enrichment markers	Flow sorting subsets (examples)	Reagents for positive selection of desired cells	Reagents for depletion of unwanted cells
CD45	T cells: CD45 ⁺ CD3 ⁺ CD19 ⁻ B cells: CD45 ⁺ CD19 ⁺ CD3 ⁻	CD45 MicroBeads 130-045-801	
CD3	T helper cells: CD3 ⁺ CD4 ⁺ CD8 ⁻ Cytotoxic T cells: CD3 ⁺ CD8 ⁺ CD4 ⁻	CD3 MicroBeads 130-050-101	Pan T Cell Isolation Kit 130-096-535
CD4	Naive T cells: CD4 ⁺ CD45RA ⁺ CCR7 ⁺ CD62L ⁻ CD45RO ⁻ Effector memory T cells: CD4 ⁺ CD45RO ⁺ CCR7 ⁻ CD62L ⁻ Central memory T cells: CD4 ⁺ CD45RO ⁺ CD45RA ⁻ CCR7 ⁻ CD62L ⁺ Regulatory T cells: CD4 ⁺ CD25 ⁺ CD127 ^{lo}	CD4 MicroBeads 130-045-101	CD4 ⁺ T Cell Isolation Kit 130-096-533
CD8	Naive T cells: CD8 ⁺ CD45RA ⁺ CCR7 ⁺ CD62L ⁻ CD45RO ⁻ Effector memory T cells: CD8 ⁺ CD45RO ⁺ CCR7 ⁻ CD62L ⁻ Central memory T cells: CD8 ⁺ CD45RO ⁺ CD45RA ⁻ CCR7 ⁻ CD62L ⁺	CD8 MicroBeads 130-045-201	CD8 ⁺ T Cell Isolation Kit 130-096-495
CD19	Naive B cells: CD19 ⁺ IgD ⁺ CD27 ⁻ Memory B cells: CD19 ⁺ CD27 ⁺ Ig D/A ⁺	CD19 MicroBeads 130-050-301 REAl ease™ CD19 MicroBead Kit 130-117-034	Pan B Cell Isolation Kit 130-101-638
CD11b	M-MDSC: CD11b ⁺ CD15 ⁻ CD14 ⁻ CD33 ⁺ HLADR ^{lo} G-MDSC: CD11b ⁺ CD15 ⁺ CD56b ⁺	CD11b MicroBeads 130-049-601	
CD34	HSC: CD34 ⁺ CD38 ⁻ Thy-1 ⁺ CD45RA ⁻ Flt3 ⁺ CD7 ⁻ CD10 ⁻	CD34 MicroBead Kit UltraPure 130-100-453	Lineage Cell Depletion Kit 130-092-211

Selected cell separation reagents for pre-enrichment of mouse cells

Pre-enrichment markers	Flow sorting subsets (examples)	Reagents for positive selection of desired cells	Reagents for depletion of unwanted cells
CD45	T cells: CD45 ⁺ CD3 ⁺ CD19 ⁻ B cells: CD45 ⁺ CD19 ⁺ CD3 ⁻	CD45 MicroBeads 130-052-301	
CD3	T helper cells: CD3 ⁺ CD4 ⁺ CD8 ⁻ Cytotoxic T cells: CD3 ⁺ CD8 ⁺ CD4 ⁻	CD3ε MicroBead Kit 130-094-973	Pan T Cell Isolation Kit 130-096-535
CD4	Naive T cells: CD4 ⁺ CD44 ^{lo} CD62L ⁺ Regulatory T cells: CD4 ⁺ CD25 ⁺ Foxp3 ^{**}	CD4 (L3T4) MicroBeads 130-117-043	CD4 ⁺ T Cell Isolation Kit 130-104-454
CD8	Naive T cells: CD8 ⁺ CD44 ^{lo} CD62L ⁺	CD8a (Ly-2) MicroBeads 130-117-044	CD8 ⁺ T Cell Isolation Kit 130-104-075
CD19 B220 (CD45RA)	Naive B cells: CD19/B220 ⁺ IgM ⁺ IgD ⁺ CD23 ⁺ Follicular B cells: CD19/B220 ⁺ IgM ^{lo} IgD ^{hi} CD21 ^{int} CD23 ⁺ Marginal B cells: CD19/B220 ⁺ IgM ⁺ IgD ^{lo} CD1d ^{hi} CD21 ^{hi} CD23 ⁻	CD19 MicroBeads 130-052-201 CD45R (B220) MicroBeads 130-049-501	Pan B Cell Isolation Kit II 130-104-443
CD11c	CD11b ⁺ cDC: CD11c ⁺ MHCII ⁺ CD11b ^{hi} CD172a ⁺ XCR1 ⁺ cDC: CD11c ⁺ MHCII ⁺ CD103 ⁺ XCR1 ⁺	CD11c MicroBeads UltraPure 130-108-338	Pan DC Isolation Kit 130-100-875
Lineage depletion	HSC: Lin ⁻ Sca-1 ⁺ c-kit ⁺ CD48 ⁻ CD150 ⁺		Direct Lineage Cell Depletion Kit 130-110-470

*FoxP3 is an intracellular marker. Cells from FoxP3 reporter mice are suitable for flow sorting.

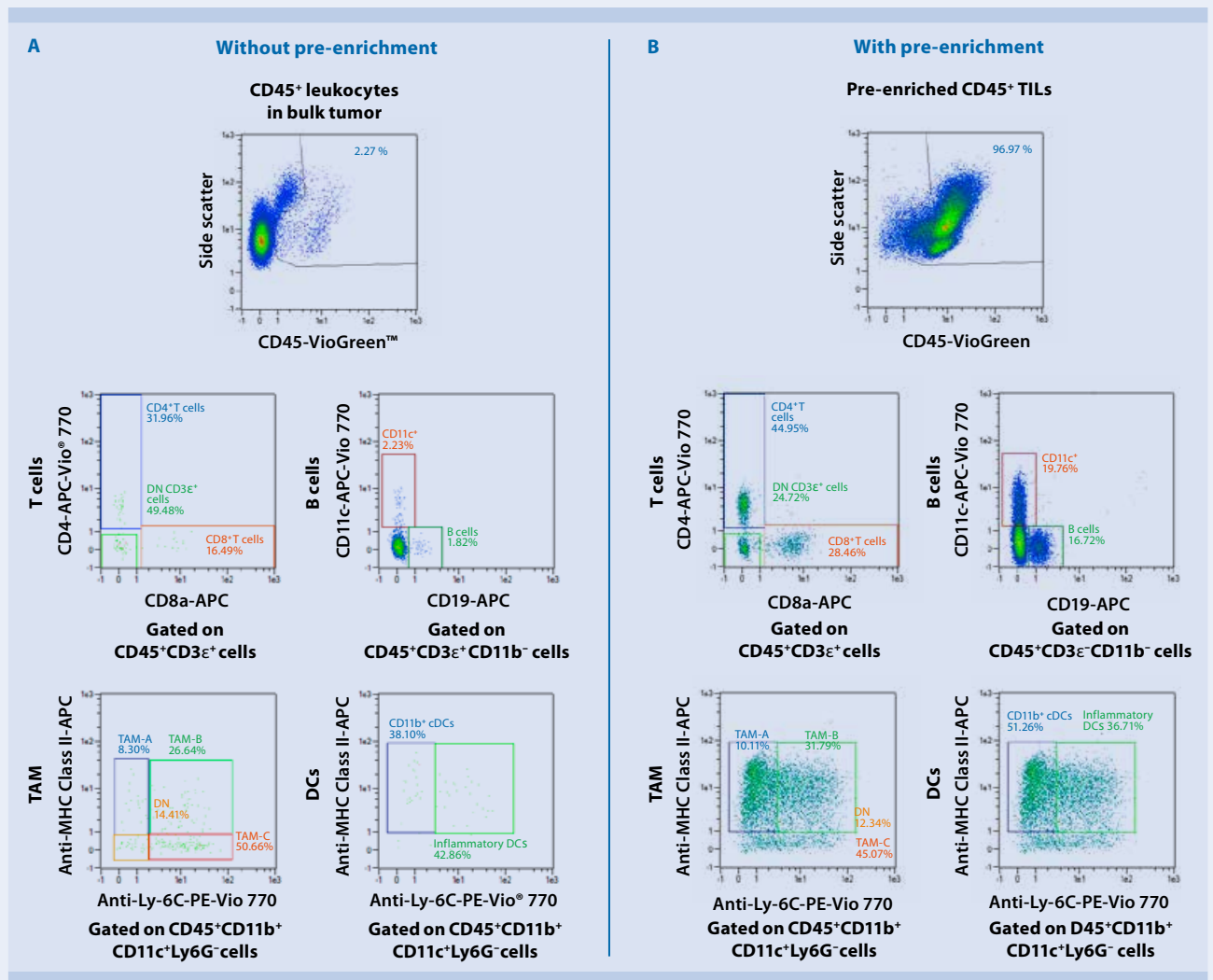
MACS® Cell Pre-Enrichment for flow analysis

Enhanced quality of flow cytometry data

Improving the analysis of rare cell populations

Analyzing rare populations such as tumor-infiltrating leukocytes or lymphocytes (TILs) with cell analyzers is often challenging, as these small target populations can easily be lost in the background noise. Obtaining a number of events that is large enough for a detailed and significant analysis of subpopulations can be very time consuming, especially when analyzing multiple samples.

MACS Technology enables pre-enrichment of cells prior to analysis in quick and easy steps. Pre-enriched samples can then be directly analyzed by flow cytometry in a shorter time and a more detailed manner, which ultimately results in an increased quality of flow cytometry data.



MACS® Cell Pre-Enrichment for flow analysis

Getting reliable flow cytometry data faster

Cell pre-enrichment speeds up cell analysis

It takes quite some time to acquire enough cells for the accurate flow cytometry analysis of a rare subpopulation. Enrichment of the cells prior to analysis however minimizes

the time to get significant flow cytometry data. Particularly when analyzing large numbers of samples the total analysis time can be reduced considerably.

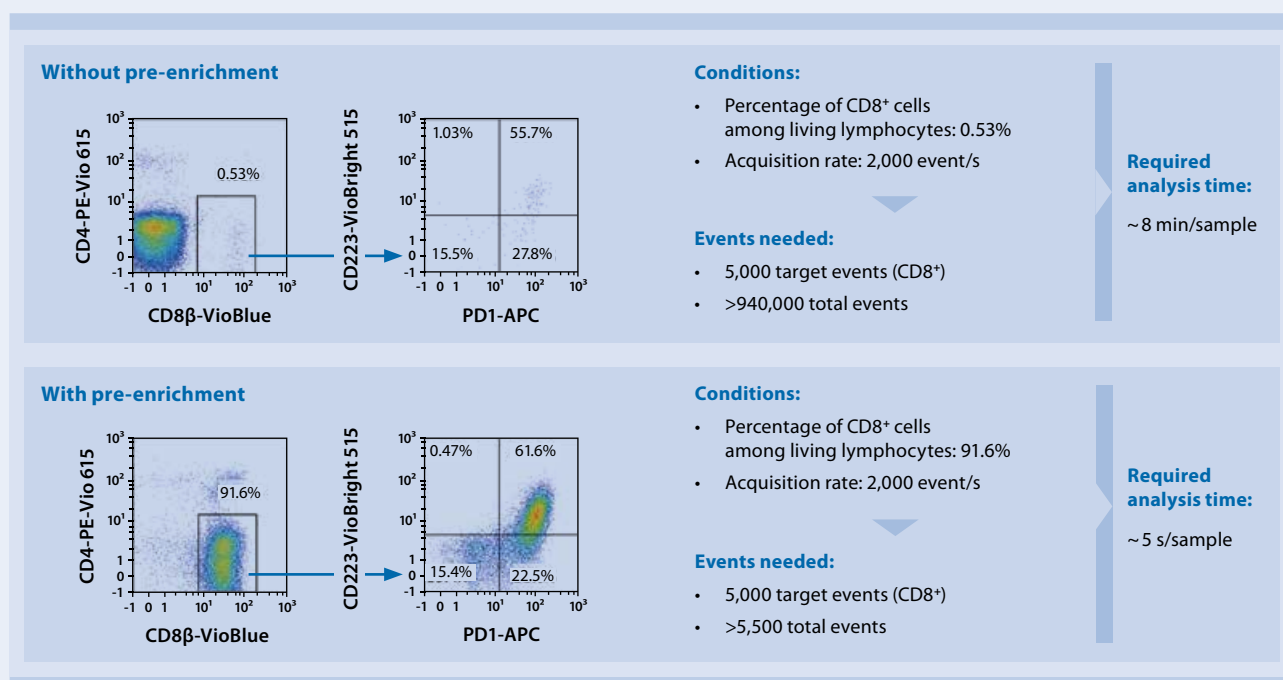


Figure 4: Example of time calculation for flow cytometry analysis of TILs. Cells derived from B16-F10 tumors were analyzed by flow cytometry with or without pre-enrichment using CD8 (TIL) MicroBeads. Cells were stained with REAfinity™ Antibodies CD4-PE-Vio® 615, CD8β-VioBlue®, CD223-VioBright™ 515, and PD1-APC. Theoretical acquisition time is calculated based on achieving 5,000 target events.

Estimated time required to acquire specified TIL populations

Target population	CD45 ⁺ cells		CD4 ⁺ T cells		CD8 ⁺ T cells		Pan T cells	
	Bulk	Pre-enriched*	Bulk	Pre-enriched*	Bulk	Pre-enriched*	Bulk	Pre-enriched*
Target events	5,000	5,000	5,000	5,000	5,000	5,000	10,000	10,000
Total collected events	5×10 ⁵	2×10 ⁴	7.9×10 ⁶	5.4×10 ⁴	2.8×10 ⁶	4.4×10 ⁴	8.1×10 ⁵	3.2×10 ⁴
Acquisition time/sample**	4 min	0.2 min	66 min	0.5 min	23 min	0.4 min	7 min	0.3 min
Total acquisition time for 12 samples***	~1 h	<10 min	>10 h	~11 min	>3.5 h	<10 min	>1 h	<10 min

*Isolation using CD45 (TIL) MicroBeads, CD8 (TIL) MicroBeads, CD4 (TIL) MicroBeads, or CD4/CD8 (TIL) MicroBeads, respectively.

** Acquisition rate: 2,000 events/s on MACSQuant® Analyzer 10. *** Includes 45 s automated mixing and rinsing between samples.

Selected products for TIL pre-enrichment

- CD45 (TIL) MicroBeads, human (# 130-118-780)
- CD45 (TIL) MicroBeads, mouse (# 130-110-618)
- CD4/CD8 (TIL) MicroBeads, mouse (# 130-116-480)
- CD4 (TIL) MicroBeads, mouse (# 130-116-475)
- CD8 (TIL) MicroBeads, mouse (# 130-116-478)

Seamless flow compatibility of MACS® Technology

Flexible, effortless cell pre-enrichment strategies

Fast and easy cell pre-enrichment with MACS® MicroBead Technology

Enriching cells for subsequent sorting is accomplished in three straightforward steps. MACS Cell Pre-Enrichment ensures you maximum efficiency and flexibility:

- Elimination of 20–80% of unwanted cells
- No removal of MACS MicroBeads necessary as they are fully compatible with flow sorting
- Target cells can be enriched by positive selection or depletion of unwanted cells

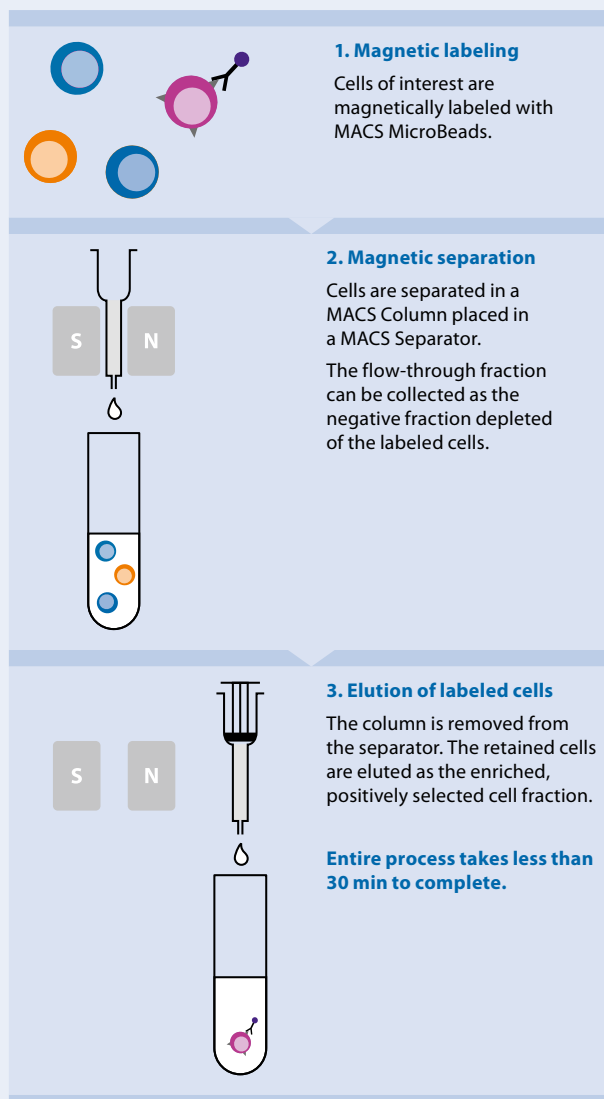


Figure 5: Principle of MACS MicroBead Technology.

Automated cell pre-enrichment solution: autoMACS® Pro Separator

The autoMACS Pro Separator pre-enriches cells in a fully automated fashion. The instrument is compatible with hundreds of MACS Cell Separation Reagents for effective isolation of virtually any cell type from any species. At the heart of the autoMACS Pro Separator is MACS MicroBead Technology, which makes it the perfect match for flow sorting.

- Minimal hands-on time with fully automated cell labeling and separation
- True walk-away cell separation with sensor-controlled processes, including startup and housekeeping
- Flexible, easy-to-use benchtop instrument for a multi-user environment



Figure 6: autoMACS Pro Separator – fully automated cell separation for true walk-away convenience.

LEARN MORE

Learn more details about MACS Cell Pre-Enrichment
▶ miltenyibiotec.com/preenrichment

Find out about indirect magnetic labeling, providing the option to pre-enrich any cell type
▶ miltenyibiotec.com/indirect-labeling

Automated MACS® Cell Pre-Enrichment

Streamlining flow sorting and analysis with high-throughput MACS® Cell Separation

MultiMACS™ Cell24 Separator Plus

The MultiMACS Cell24 Separator Plus was specifically developed for simultaneous multisample magnetic cell separations.

- Functional design for large sample numbers or volumes
- Convenient and flexible handling with touchscreen interface
- Compatible with any starting material and cell separation strategy



Figure 7: MultiMACS Cell24 Separator Plus – semi-automated and flexible for easy multisample processing.

MultiMACS™ X

The MultiMACS X is a high-throughput instrument for laboratories requiring fully automated processing of large sample numbers or sample volumes.

- Fully automated magnetic cell labeling and separation
- Maximal reproducibility through parallel processing of up to 24 samples
- Customized processes ensure the perfect match with any workflow



Figure 8: MultiMACS X – full automation from start to finish with integrated liquid handling system.

Instrument	Order no.
autoMACS Pro Separator – Starter Kit	130-092-545
MultiMACS Cell24 Separator Plus	130-098-637
MultiMACS X	130-118-515

LEARN MORE



Find more information about automated
MACS Cell Separation

► miltenyibiotec.com/macs-automation

Powerful flow sorting and cell analysis

From MACS® Cell Pre-Enrichment to high-speed cell sorting and analysis

Revolutionary benchtop high-speed cell sorting: MACSQuant® Tyto®

The MACSQuant Tyto is revolutionizing cell sorting: Patented microchip-based technology enables high-speed, 10-parameter sorting in a fully closed cartridge system, the MACSQuant Tyto Cartridge. With its easy “plug-and-play” format and fully automated laser alignment, the MACSQuant Tyto makes cell sorting accessible to any lab professional. Additionally, the closed Tyto Cartridge provides full operator safety and protects the cell sample from contamination.

- Microchip-based cell sorting facilitates gentle processing
- High-speed multiparameter flow sorting in the safety of a fully enclosed cartridge system
- Simple loading, automated sort setup, and operator-free sorting



Figure 9: MACSQuant Tyto – high-speed multiparameter flow sorting in the safety of a fully closed cartridge system.

Advanced multiparameter cell analysis: MACSQuant® Flow Cytometers

MACSQuant Analyzer 10, MACSQuant Analyzer 16, MACSQuant VYB, and MACSQuant X are powerful benchtop instruments for highly sensitive, multiparameter cell analysis.

- 3 lasers, up to 14 colors plus two scatter channels for multiparameter flow cytometry
- Integrated multisample analysis of up to 384 wells
- Autolabeling function and MACS Column for magnetic rare cell enrichment



Figure 10: MACSQuant Flow Cytometers – ultra-compact instruments combining multisample and multiparameter analysis with unrivaled ease of use.

Instrument	Order no.
MACSQuant Tyto	130-103-931
MACSQuant Analyzer 10	130-096-343
MACSQuant VYB	130-096-116
MACSQuant X	130-105-100

LEARN MORE 

Find out about MACSQuant Tyto and MACSQuant Flow Cytometers

- ▶ [miltenyibiotec.com/tyto](https://www.miltenyibiotec.com/tyto)
- ▶ [miltenyibiotec.com/flow-cytometers](https://www.miltenyibiotec.com/flow-cytometers)

Automated MACS® Cell Pre-Enrichment



Quick



Simple



Safe



► miltenyibiotec.com/preenrichment



Miltenyi Biotec

**Germany/Austria/
Switzerland**
Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone +49 2204 8306-0
Fax +49 2204 85197
macs@miltenyibiotec.de

USA/Canada
Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn, CA 95602, USA
Phone 800 FOR MACS
Phone +1 530 888 8871
Fax +1 877 591 1060
macs@miltenyibiotec.com

Australia
Miltenyi Biotec
Australia Pty. Ltd.
Unit 16A, 2 Eden Park Drive
Macquarie Park NSW 2113
Australia
Phone +61 2 8877 7400
Fax +61 2 9889 5044
macs@miltenyibiotec.com.au

Benelux
Miltenyi Biotec B.V.
Schipholweg 68 H
2316 XE Leiden
The Netherlands
macs@miltenyibiotec.nl
**Customer service
The Netherlands**
Phone 0800 4020120
Fax 0800 4020100

Customer service Belgium
Phone 0800 94016
Fax 0800 99626
Customer service Luxembourg
Phone 800 24971
Fax 800 24984

China
Miltenyi Biotec Technology &
Trading (Shanghai) Co., Ltd.
Rooms 2303 and 2309
No. 319, Xianxia Road
Changning District
200051 Shanghai, P.R. China
Phone +86 21 62351005
Fax +86 21 62350953
macs@miltenyibiotec.com.cn

France
Miltenyi Biotec SAS
10 rue Mercœur
75011 Paris, France
Phone +33 1 56 98 16 16
Fax +33 1 56 98 16 17
macs@miltenyibiotec.fr

Italy
Miltenyi Biotec S.r.l.
Via Paolo Nanni Costa, 30
40133 Bologna
Italy
Phone +39 051 6 460 411
Fax +39 051 6 460 499
macs@miltenyibiotec.it

Japan
Miltenyi Biotec K.K.
Nittsu-Eitai Building 5F
16-10 Fuyuki, Koto-ku,
Tokyo 135-0041, Japan
Phone +81 3 5646 8910
Fax +81 3 5646 8911
macs@miltenyibiotec.jp

Nordics and Baltics
Miltenyi Biotec Norden AB
Scheelevägen 17
223 70 Lund
Sweden
macs@miltenyibiotec.se
Customer service Sweden
Phone 0200-111 800
Fax 046-280 72 99

Customer service Denmark
Phone 80 20 30 10
Fax +46 46 280 72 99
**Customer service
Norway, Finland, Iceland,
and Baltic countries**
Phone +46 46 280 72 80
Fax +46 46 280 72 99

Singapore
Miltenyi Biotec Asia Pacific Pte Ltd.
100 Beach Road
#28-06 to 28-08 Shaw Tower
Singapore 189702
Phone +65 6238 8183
Fax +65 6238 0302
macs@miltenyibiotec.com.sg

South Korea
Miltenyi Biotec Korea Co., Ltd
Arigi Bldg. 8F
562 Nonhyeon-ro
Gangnam-gu
Seoul 06136, South Korea
Phone +82 2 555 1988
Fax +82 2 555 8890
macs@miltenyibiotec.co.kr

Spain
Miltenyi Biotec S.L.
C/Luis Buñuel 2
Ciudad de la Imagen
28223 Pozuelo de Alarcón (Madrid)
Spain
Phone +34 91 512 12 90
Fax +34 91 512 12 91
macs@miltenyibiotec.es

United Kingdom
Miltenyi Biotec Ltd.
Almac House, Church Lane
Bisley, Surrey GU24 9DR, UK
Phone +44 1483 799 800
Fax +44 1483 799 811
macs@miltenyibiotec.co.uk

www.miltenyibiotec.com

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