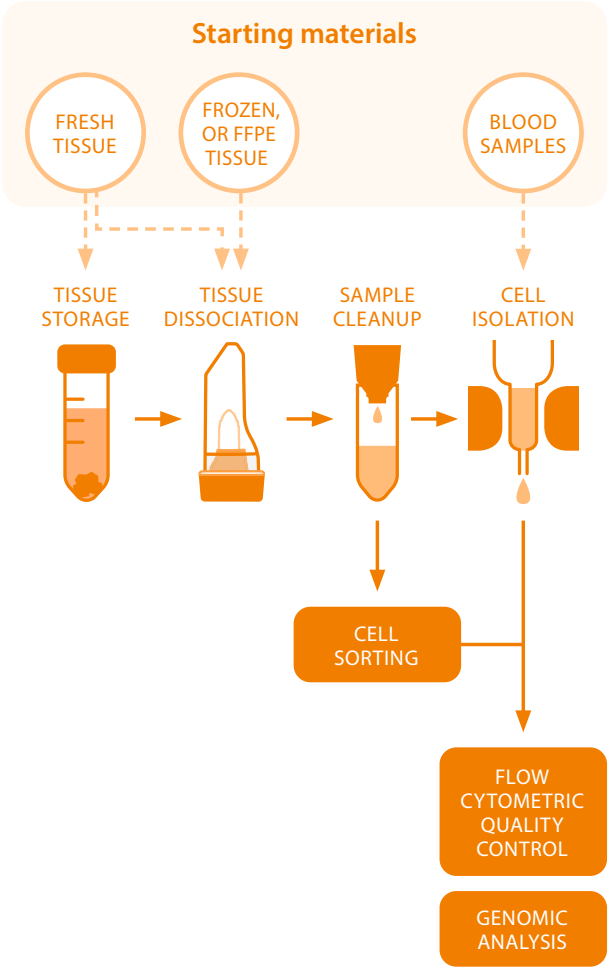
Miltenyi Biotec

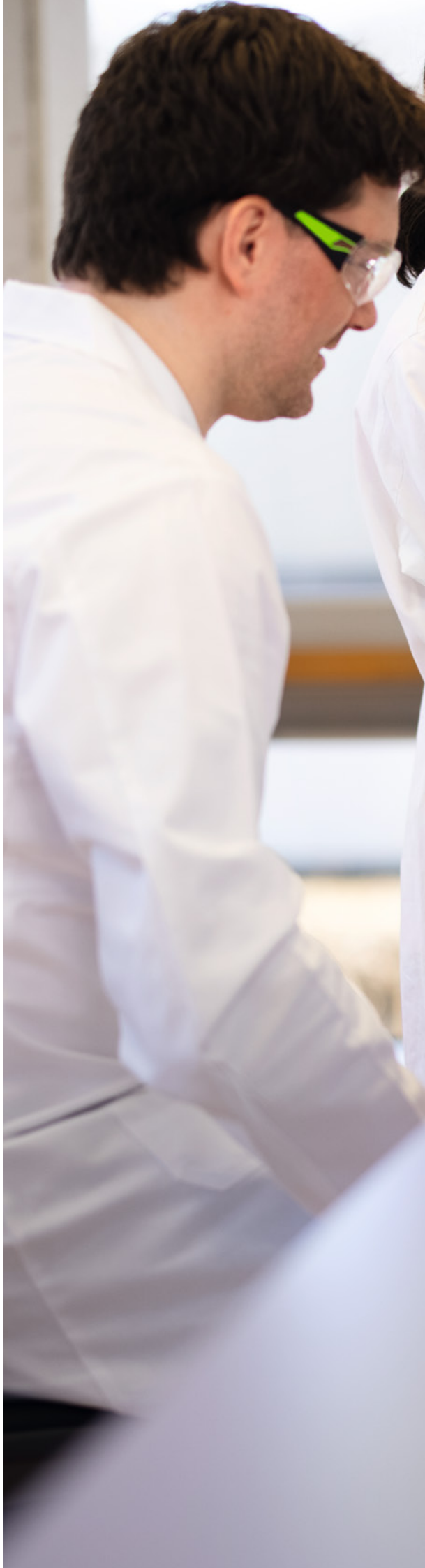
Cell preparation for genomics

Start smart to achieve new heights
in genomic analyses

Improved sample quality makes the difference

We have a solution for every step in the preparation of cells and nuclei for excellent genomic analysis results. From tissue storage to quality control, our technology will help your research achieve new heights.





Contents

Tissue storage, dissociation, and cleanup

The preparation of viable single-cell suspensions or intact nuclei suspensions is crucial when performing single-cell genomic analyses. Our sample preparation products ensure efficient cell and nuclei preparation and offer flexibility for fresh, frozen, or FFPE tissue processing.

Tissue storage

Short-term tissue storage helps you to gain flexibility in sample processing. The MACS® Tissue Storage Solution allows the optimal storage of fresh solid tissues validated for up to 48 hours at 2–8 °C. It has been tested with a variety of human and rodent tissues including tumor, lung, spleen, brain, or skeletal muscle. Storing tissues in MACS Tissue Storage Solution prevents the induction of necrosis and cellular stress while preserving the cellular composition and the activation status of cells.

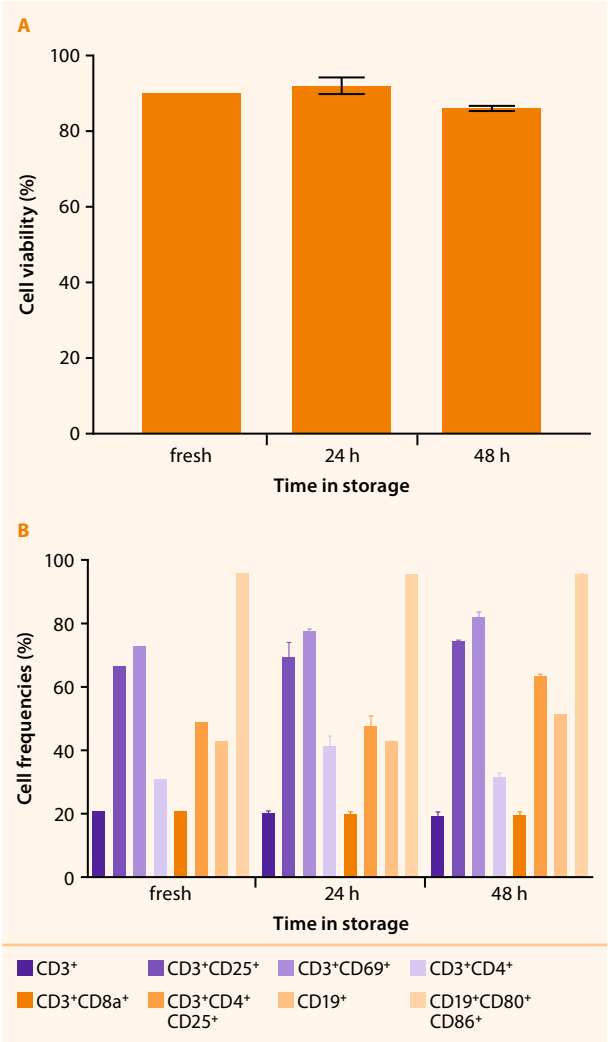


Figure 1: Preserved cell viability and TIL composition of mouse tumors stored in MACS Tissue Storage Solution. CT26 mouse tumors were stored at 4 °C in MACS Tissue Storage Solution for 24 or 48 h. After dissociation, total cell viability (A) and TILs (tumor infiltrating leukocytes) composition (B) were analyzed by flow cytometry. Cell frequencies are referred to viable CD45+ cells. Additionally, frequencies of CD3+ and CD19+ cell subsets refer to corresponding parental CD3+ and CD19+ populations respectively.

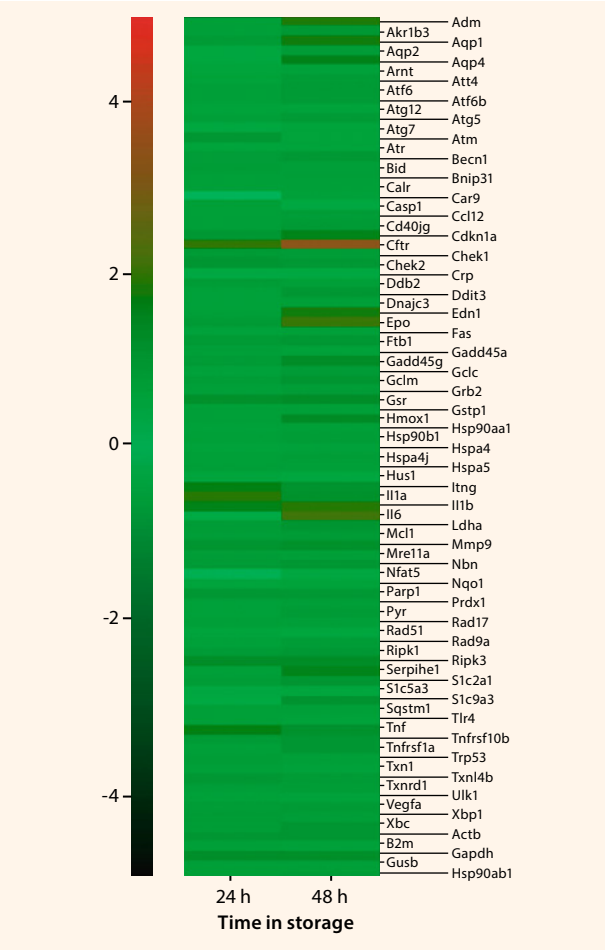


Figure 2: Expression of cellular stress-related genes in cells from fresh and stored tumors. RNA was isolated from fresh and stored cells from CT26 tumors and expression of cellular stress-related genes was analyzed. The heat map shows the fold-change of expression rates compared to fresh tissue.

Preparation of cells from solid tissues

The efficient dissociation of solid tissues into viable single-cell suspensions is the most critical step in the single-cell genomics workflow. The cellular composition of the tissue must be preserved after dissociation, as well as cell surface epitopes to further isolate specific target cells. Our gentleMACS™ Technology enables the easy and fast dissociation of solid tissues in a closed and sterile system that combines mechanical disruption and enzymatic digestion to be as gentle to cells as possible.

gentleMACS Dissociators and Tubes

The gentleMACS Octo Dissociator with Heaters allows for fully automated tissue dissociation. This dissociator has eight sample positions with integrated heating units to perform enzymatic incubation directly on the instrument. Each position can run independently, enabling you to process samples at any time, even if other positions are being used. Over 40 pre-defined programs have been developed along with our tissue dissociation kits for optimal results. Moreover, customized programs can be created to meet your specific needs.

The gentleMACS Tube is an integral component of gentleMACS Technology. Every element of the tube has been engineered to ensure the highest performance in the dissociation or homogenization of tissue samples. Purple capped C Tubes are used for tissue dissociation to get viable single-cell suspensions from tissues, whereas orange capped M Tubes are used for thorough tissue homogenization for subsequent molecular analysis.

MACS® Tissue Dissociation Kits

We offer a wide variety of dissociation kits for human and rodent tissues. Our kits have lot-to-lot consistency and contain highly-purified enzymes that ensure standardized and reproducible dissociation results with preserved cellular epitopes. For all dissociation kits, we provide pre-defined protocols to ensure the maximal recovery of viable cells.

Analysis of mouse tumor heterogeneity at the single-cell level

Optimal dissociation of mouse tumors leads to the clear identification of different cellular populations present in the tissue by single-cell gene expression analysis.

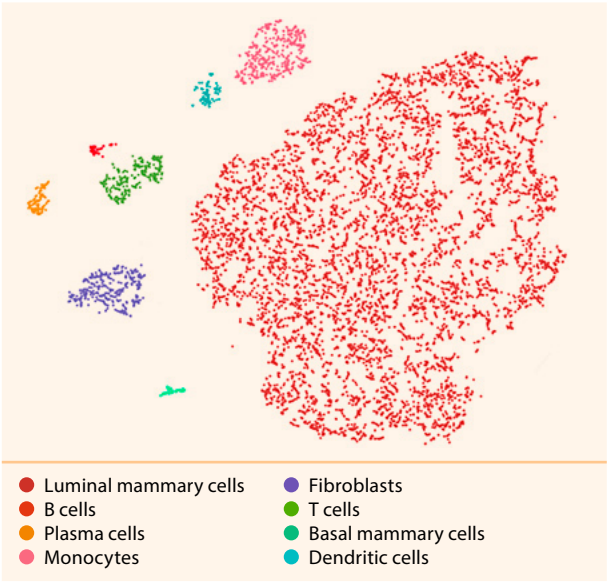


Figure 3: Single-cell gene expression analysis of mammary mouse tumors. Tumors were dissociated using the Tumor Dissociation Kit, mouse in combination with the gentleMACS Octo Dissociator with Heaters. Single-cell RNAseq libraries were generated using the Chromium™ Platform from 10x Genomics, and sequenced using Illumina's technology. Clustering was performed using Cell Ranger™ Software and visualized in Loupe Cell Browser, both from 10x Genomics. Cell type classification was added to "graph-based" clusters based on manual curation using known marker genes.

Preparation of nuclei from solid tissues

Some genomic applications require nuclei as starting material. Also for certain tissues like snap-frozen tissues, nuclei extraction is the only option for the analysis, since cells are heavily damaged during the thawing process. For these applications we have developed the Nuclei Extraction Buffer. Together with the gentleMACS™ Octo Dissociators and C Tubes, the Nuclei Extraction Buffer enables the fast extraction of single nuclei at 4 °C within seven minutes from up to eight samples in parallel. The Nuclei Extraction Buffer has been successfully tested with a wide variety of fresh and frozen tissues, including mouse brain, liver, heart and kidney, mouse xenograft tumors, and OCT embedded primary human tumors, such as melanoma, breast, colon, and prostate.

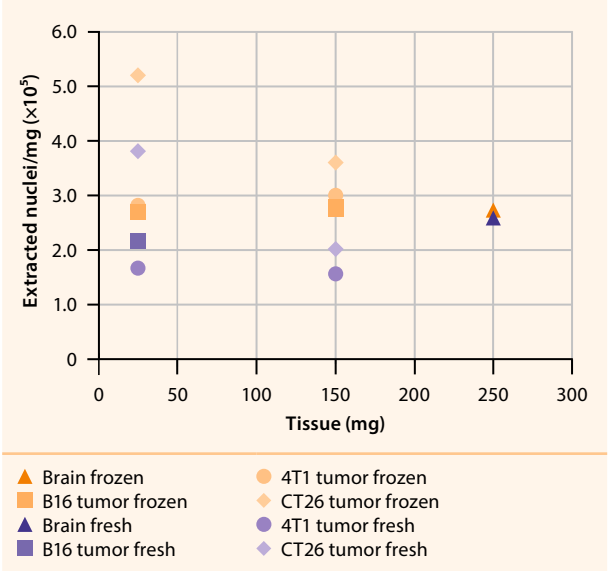


Figure 4: High yields of single nuclei after automated extraction from fresh and frozen tissues. Single-nuclei suspensions were obtained from fresh and snap-frozen mouse brain and syngeneic mouse tumors using the Nuclei Extraction Buffer and gentleMACS Octo Dissociators. Nuclei were stained with DAPI and analyzed by flow cytometry using the MACSQuant® Analyzer 10.

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Visit our website for more information on nuclei extraction:

▸ **miltenyibiotec.com/nucleiextraction**

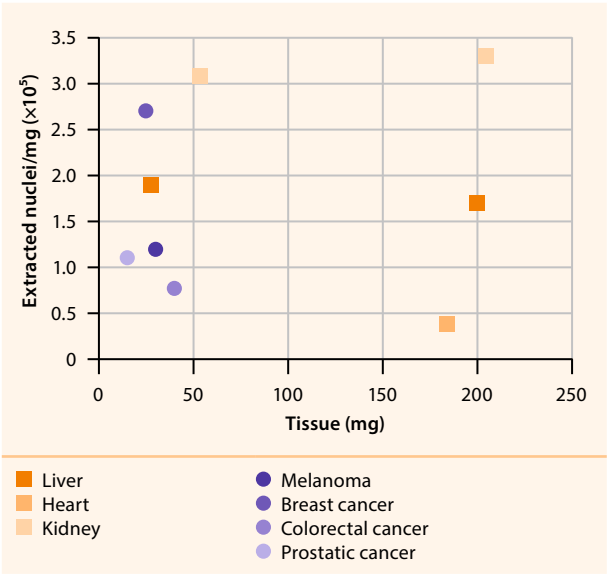


Figure 5: High yields of single nuclei after automated extraction from frozen and OCT-embedded tissues. Single-nuclei suspensions were obtained from snap-frozen mouse tissues, including liver, heart and kidney and from OCT-embedded human tumors, including melanoma, breast cancer, colorectal cancer, and prostatic cancer by using the Nuclei Extraction Buffer in combination with the gentleMACS Octo Dissociator. Nuclei were stained with DAPI and analyzed by flow cytometry using the MACSQuant Analyzer 10.

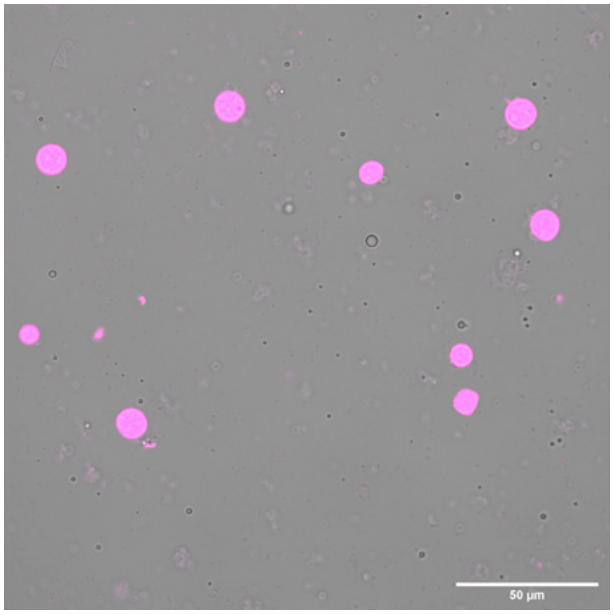


Figure 6: Single-nuclei suspension from mouse liver. Single nuclei were extracted from snap-frozen mouse liver using the Nuclei Extraction Buffer in combination with the gentleMACS Octo Dissociator. Immediately after nuclei extraction, nuclei were stained using DRAQ5™ Staining Solution. The image shows an overlay of DRAQ5™ (pink) and brightfield.

Sample cleanup

Cell suspensions often contain unwanted material, such as cell aggregates, dead cells, debris, and red blood cells, which can interfere with proper cell count and the results of your genomic downstream application. When performing single-cell transcriptomic analysis, only viable cells will generate reliable genomic information.

Our MACS® SmartStrainers and removal reagents efficiently clean and prepare your samples for genomic analysis.

With their special design, MACS SmartStrainers enable filtration without clogging and easily fit onto 15 mL and 50 mL tubes for the fast removal of larger particles and cell aggregates in cell suspensions or blood samples.

Sequence viable nucleated cells and ensure proper cell count

Dead cells, red blood cells, and debris can hamper your genomic analysis and should therefore be removed from the sample.

Use the Dead Cell Removal Kit for the fast and straightforward magnetic depletion of dead cells from peripheral blood mononuclear cells (PBMCs), cryopreserved cells, or cell suspensions from solid tissues containing robust cells, such as epithelial cells, tumor cells, and immune cells.

The Debris Removal Solution is a ready-to-use density-gradient reagent that allows the fast and convenient removal of debris in cell suspensions containing fragile cells, including cells from adult mouse brain, heart, liver, and kidney. Both dead cell or debris removal, in combination with red blood cell lysis, provide the perfect solution to increase the yield of viable target cells for downstream applications.

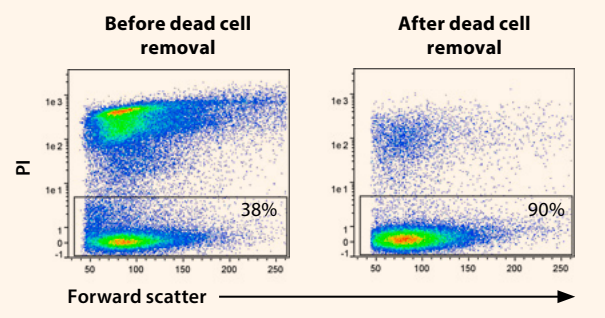


Figure 7: Efficient removal of dead cells even from samples with high dead cell content. The Dead Cell Removal Kit was used to remove dead cells from PBMC samples. Dead cells are defined as PI-positive cells and viable cells as PI-negative.

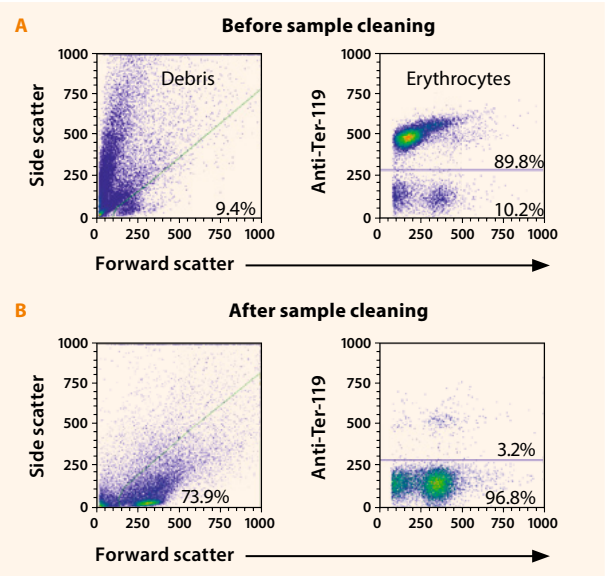


Figure 8: Enrichment of highly viable neural cells from adult mouse brain. (A) The cell suspension from adult brain contains a significant amount of cell debris and erythrocytes. (B) Viable neural cells are enriched after efficient removal of debris and erythrocytes using the Debris Removal Solution and Red Blood Cell Lysis Solution (10×).

Cleanup of tumor samples improves the quality of single-cell genomic analysis

Effective filtration and removal of dead cells and red blood cells prior to single-cell sequencing of mouse tumors helps to increase the cell recovery rate, leading to higher sequencing data quality.

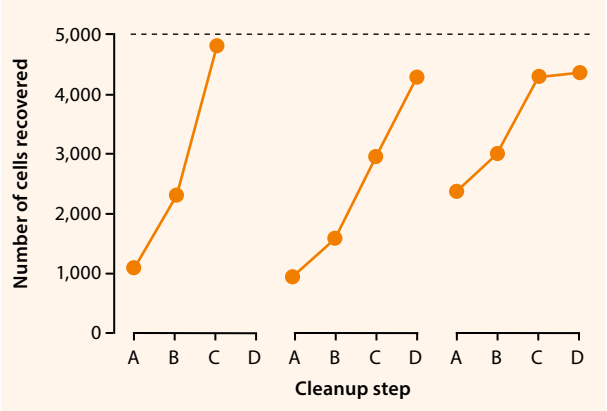


Figure 9: Impact of sample cleanup in single-cell RNA sequencing of three mouse colon tumor replicates. Tumors were dissociated with gentleMACS™ Octo Dissociator with Heaters and Tumor Dissociation Kit, mouse followed by filtration with a 70 µm MACS® SmartStrainer (A); red blood cells were removed with Red Blood Cell Lysis Solution (10×) (B); and dead cells were removed once (C) or twice (D) using the Dead Cell Removal Kit. Aliquots were taken at each step and single-cell RNAseq libraries were generated with the Chromium™ Platform from 10x Genomics and sequenced using Illumina's technology. Data was analyzed using Cell Ranger™ software from 10x Genomics.

Single-cell gene expression analysis of adult mouse brain

Optimized dissociation of adult mouse brain, including debris removal and red blood cell lysis, allows for the efficient single-cell sequencing analysis of major neural cell types from adult mouse brains.

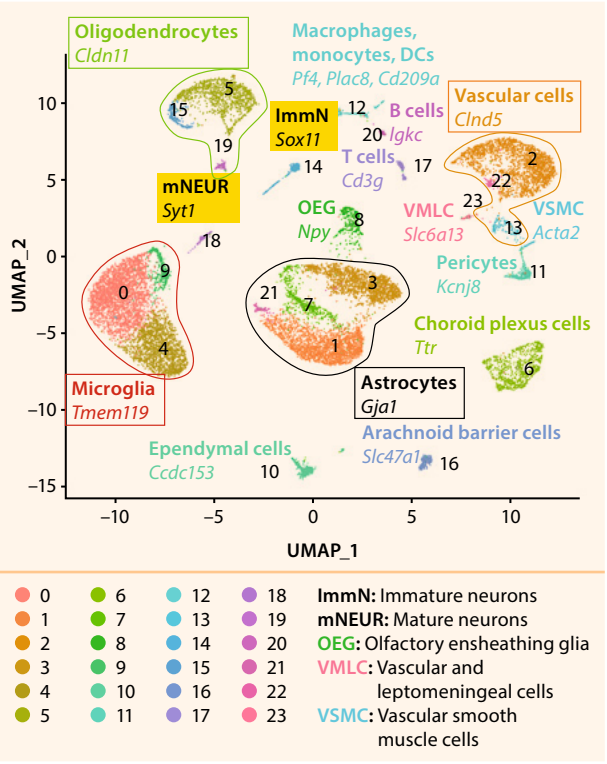


Figure 10: Single-cell gene expression analysis of adult neural cells. Whole adult mouse brains were dissociated using the Adult Brain Dissociation Kit, mouse and rat in combination with the gentleMACS Octo Dissociator with Heaters. The kit includes the reagents to perform debris and erythrocyte removal. Afterwards, cells were applied to the Chromium™ Platform from 10x Genomics and single-cell libraries were sequenced using Illumina's technology. The Uniform Manifold Approximation and Projection (UMAP) was used to visually represent the clusters of the different neural populations based on their gene expression profile.

Magnetic cell isolation

The genomic analysis of PBMCs or specific cell types from a sample can be time consuming and expensive, especially when target cells are present at low frequencies. Cell isolation helps you to increase the sensitivity of the analysis by excluding the background signals from non-target cells, while saving time and sequencing costs.

Select the best isolation approach

MACS® Technology enables the magnetic separation of cell populations by targeting surface antigens with specific antibodies conjugated to superparamagnetic beads. Labeled cells are magnetically retained in a separation column, from which they can be eluted. The fast and gentle isolation minimizes cellular changes and ensures high viability.

Based on MACS Technology, we offer different cell separation solutions to fit your every need.

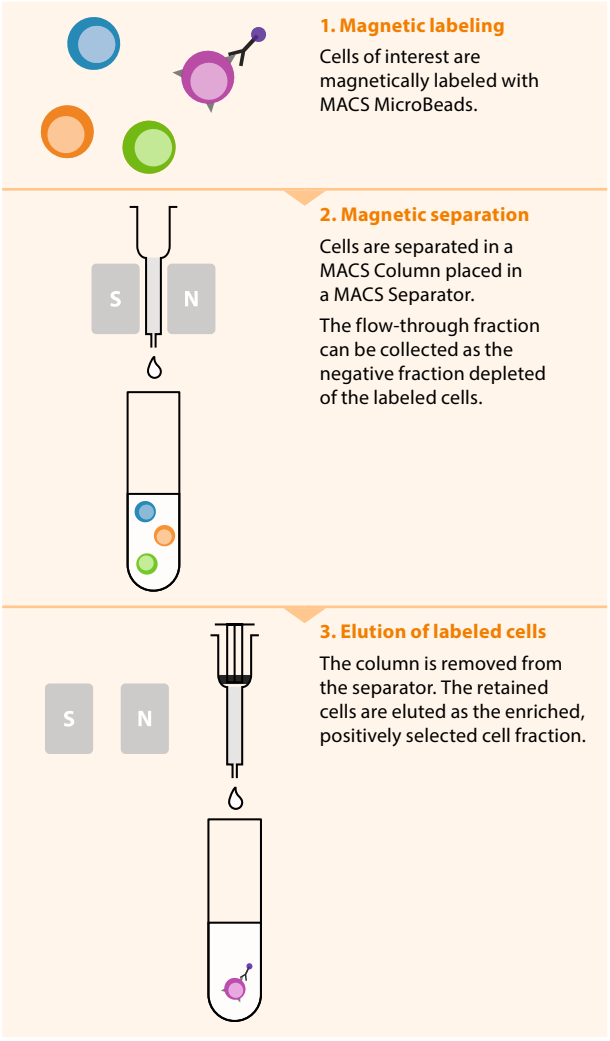
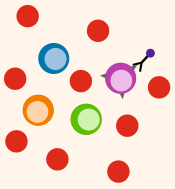


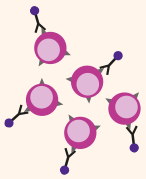
Figure 11: Three easy steps of MACS MicroBead Technology to isolate your cells of interest.

Separation solutions to fit every need



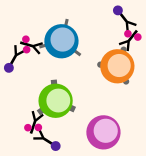
StraightFrom® MicroBeads

- Direct isolation of leukocyte subsets from blood samples in less than 30 min without density gradient centrifugation or red blood cell lysis
- Avoid cellular changes through density gradient centrifugation and red blood cell lysis



MACS MicroBeads

- Gentle, positive isolation ensures highest purity, even for rare cells
- Applicable to virtually any cell type, and tissue, from PBMCs to complex tissues
- Beads do not need to be removed for downstream applications



MACS Isolation kits

- Ideal method to isolate PBMCs and cells without a defined lineage-specific marker, such as tumor cells or neurons
- Target cells are label free and can be used for subsequent magnetic isolations

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Visit our website for more information on MACS Technology:

▸ **miltenyibiotec.com/macstech_genomics**

Automation minimizes user variation and errors

Whether you work with few samples or in a high-throughput setting, automation helps to streamline your cell separation. Our instruments for cell separation allow different levels of automation for scalable sample throughput, while standardizing cell separation processes.

For high reproducibility, the autoMACS® Pro Separator is the ideal instrument because it performs fully automated magnetic cell separation while minimizing temperature changes and user variation. Besides magnetic cell separations of particular target cells, isolation of PBMCs from blood samples and removal of dead cells using the Dead Cell Removal Kit are also possible with the autoMACS Pro.

Direct immunomagnetic isolation of PBMCs from whole blood prevents cellular stress

Gentle and automated immunomagnetic isolation of PBMCs directly from whole blood prevents activation of cellular stress responses in comparison to density gradient centrifugation methods.

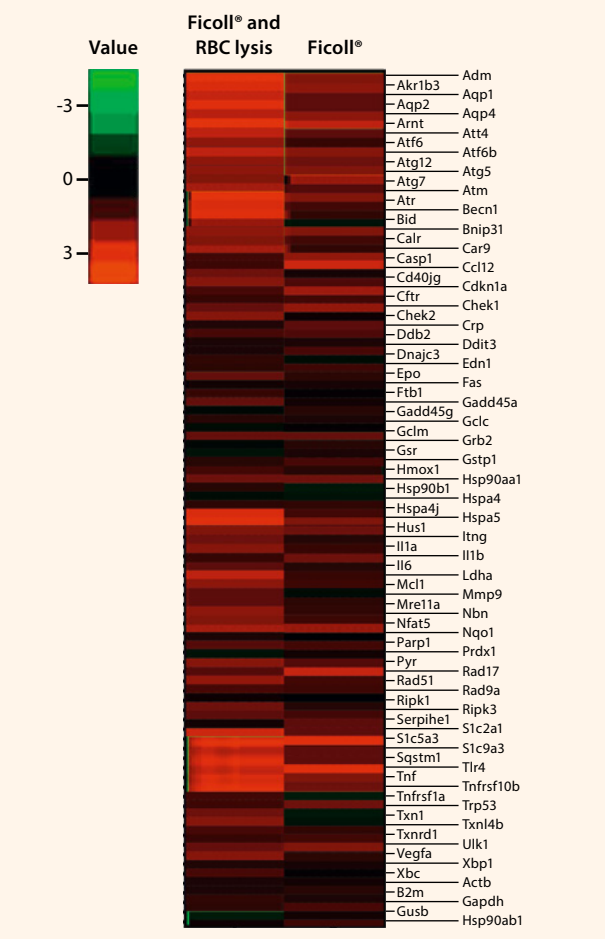


Figure 12: Immunomagnetic-based isolation of PBMCs from whole blood prevents cellular stress. PBMCs were prepared using density gradient centrifugation (Ficoll®) or using the Whole Blood PBMC Isolation Kit, human and the Sedimentation Kit 2. Red blood cells were removed using the Red Blood Cell Lysis Solution (10×) from an aliquot of the PBMCs prepared using Ficoll® (Ficoll® and RBC lysis). RNA was isolated and expression of cellular stress-related genes was analyzed. The heat map shows the fold-change of gene expression rates compared to PBMCs prepared with immunomagnetic beads.

Isolation of TILs significantly increases the sensitivity of single-cell immune profiling analysis

Magnetic isolation of TILs after tumor dissociation improves the sensitivity of single-cell RNA sequencing of T and B cell receptors, highlighting clonotypes otherwise poorly or unrepresented in the bulk sample.

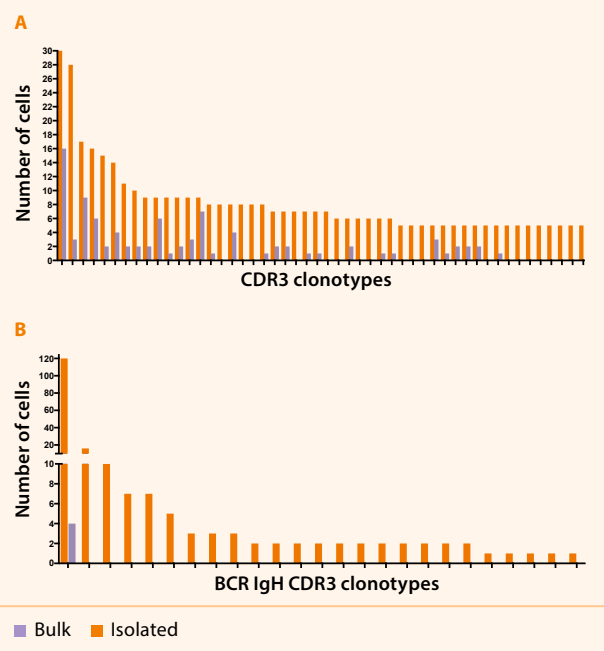


Figure 13: Isolation of TILs improves the sensitivity of single-cell immune profiling. After dissociation and cleanup of human ovarian carcinoma samples, T cells and B cells were isolated using MACS Technology. Single-cell immunoprofiling was performed on bulk samples or isolaed T and B cells using 10x Genomics' and Illumia's technologies. The graphs show the top 50 TCR (A) and top 25 BCR (B) CDR3 clonotypes ranked by their abundancy in the isolated T or B cell fraction (orange bars). The purple bars show the number of cells containing the same CDR3 clonotype of the respective receptor in the bulk sample.

Tumor cell isolation allows sensitive and robust detection of somatic mutations via NGS

Isolation of tumor cells enables accurate detection of single nucleotide polymorphisms (SNP) in human solid tumors improving detectability of loss of heterozygosity (LOH).

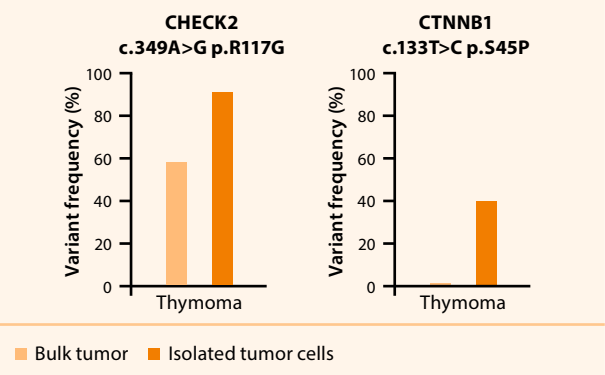
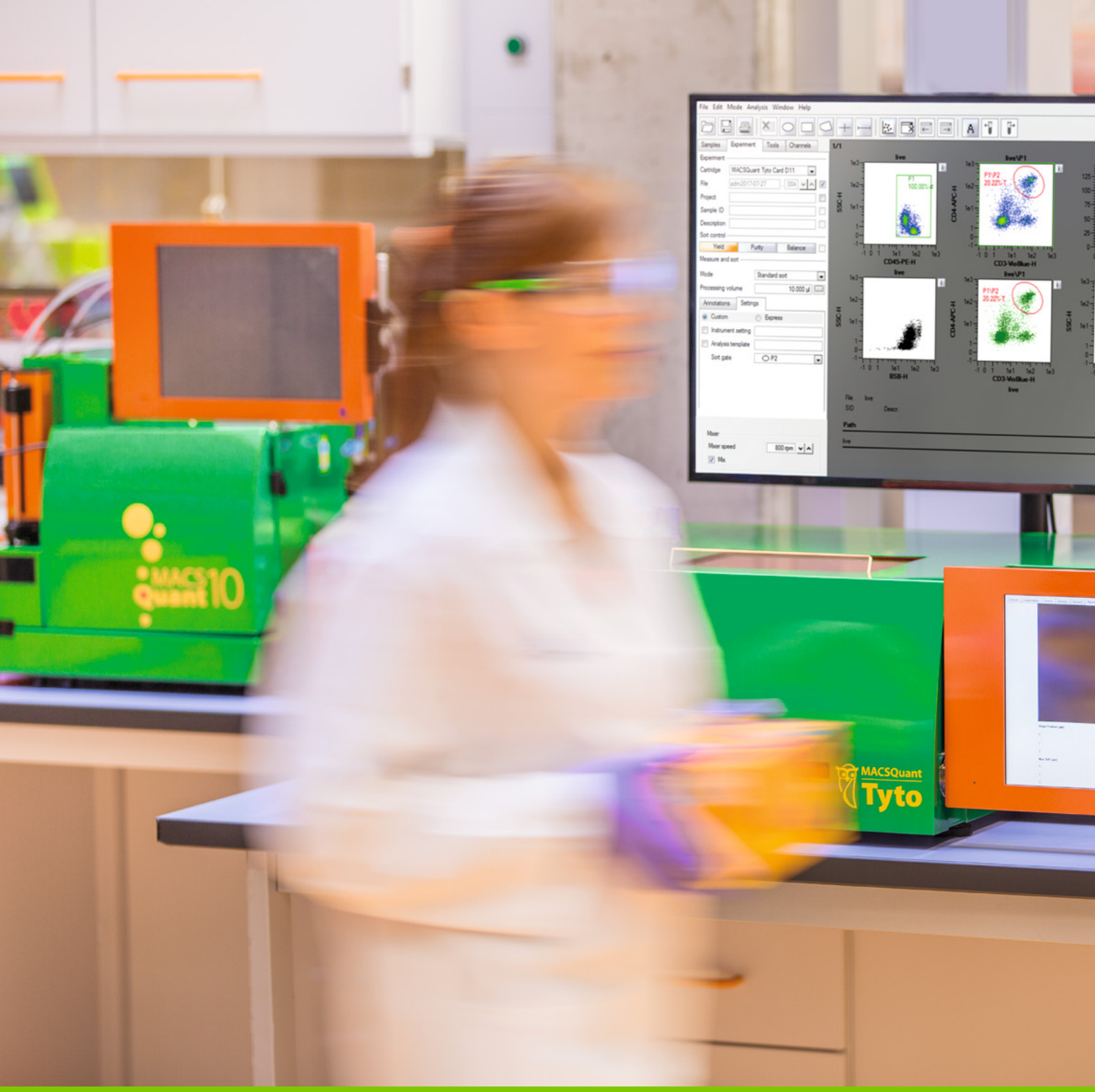


Figure 14: Tumor cell isolation enables an accurate detection of SNP zygosity in solid tumors. Tumor cells were isolated from dissociated human thymoma tumors. DNA from bulk tumor or isolated tumor cells was used to produce exome-capture sequencing libraries with Illumina's technology. The variants CHECK2 c.349A>G p.R117G and CTNNB1 c.133T>C p.S45P are depicted. While the frequency of the indicated mutation in the CHECK2 gene in the bulk tumor suggested heterozygous conditions, in the isolated tumor cell fraction LOH events could be detected. Furthermore, the somatic mutation c.133T>C p.S45P in the CTNNB1 gene could only be detected in isolated tumor cells.



Cell sorting and flow cytometric quality control

Our solutions for gentle microchip-based multiparameter cell sorting can play an important role in boosting genomic results and saving time and costs. Flow cytometric quality control can further protect against wasteful analyses and set expectations for results by providing accurate cell counts of viable cells.

Gentle multiparameter cell sorting

Flow cytometric cell sorting enables the isolation of target cells with highest purities based on the combination of different markers and physical parameters, allowing also the simultaneous depletion of dead cells and debris. However, the sorting process using droplet-based sorters can damage the cells due to high pressure and electrical charges within the system, and lead to the upregulation of stress-related genes. The MACSQuant® Tyto® Cell Sorter allows gentle multiparameter cell sorting using a unique microchip-based technology, avoiding harsh sorting conditions and thereby resulting in samples of highest quality.

The actual sorting process takes place within the MACSQuant Tyto Cartridge, a single-use and fully closed system that eliminates the risk of sample contamination and carry-over as well as the release of aerosols. In addition, during the sorting process, target cells are highly concentrated, which might supersede additional centrifugation steps after the cell sorting process.

Gentle cell sorting improves single-cell gene expression analysis of murine mammary gland epithelial cells

MACSQuant Tyto ensures the gentle cell sorting of cells, such as mammary gland epithelial cells, to minimize transcriptional changes and obtain samples of high quality to result in more reliable single-cell genomic analysis.

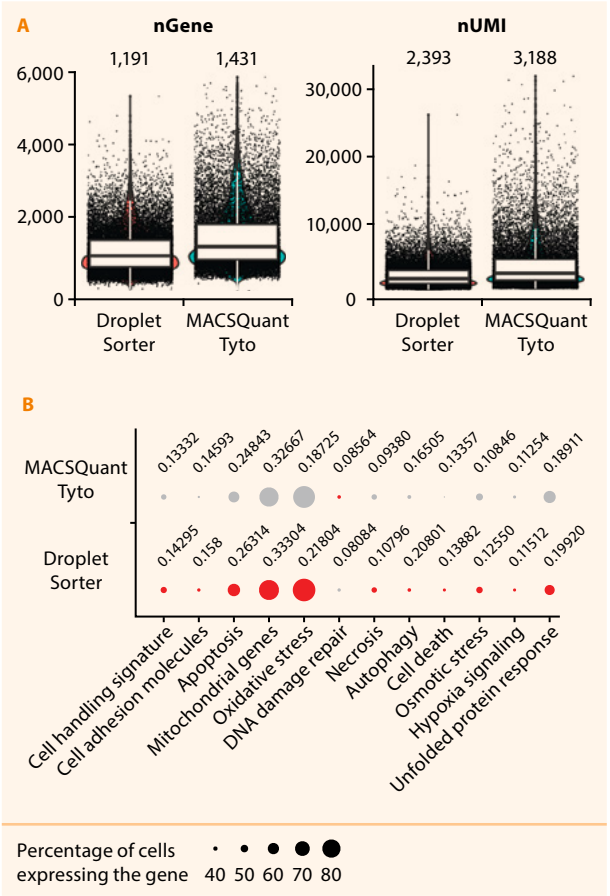


Figure 15: Gentle cell sorting improves single-cell gene expression analysis of mammary gland epithelial cells. Epithelial cells from mouse mammary glands were sorted using a droplet-based sorter or the MACSQuant Tyto. Sorted cells were then applied to the 10x Genomics Chromium™ Platform. (A) An increased number of genes and unique molecular identifiers (UMI) was obtained from cells sorted with a MACSQuant Tyto compared to a conventional droplet-based sorter. (B) Cells sorted using MACSQuant Tyto showed lower expression of stress-related genes compared to a conventional droplet-based cell sorter. Dots colored in red indicate which data set shows higher gene activation. The dot size indicates the percentage of cells expressing the respective gene. Data kindly provided by Quy Nguyen, University of California, Irvine.

Decrease the time needed for flow cytometric cell sorting and analysis

The frequency of specific cell populations in certain samples, such as tumors, can be very low and lead to extended duration of sorting and acquisition times. Pre-enrichment of these cell populations by immunomagnetic cell isolation with MACS® MicroBeads can significantly save time and increase the quality of your flow analysis or flow sorting.

Cell type	Cells to analyze	Events to collect	Flow cytometry time/sample*	Total flow cytometry time**
CD4⁺ T cells				
Bulk	5,000	7.96×10^6	66.3 min	>10 h
Isolated***	5,000	5.41×10^4	0.5 min	~11 min
CD8⁺ T cells				
Bulk	5,000	2.80×10^6	23.3 min	>3.5 h
Isolated***	5,000	4.37×10^4	0.4 min	~10 min
T cells				
Bulk	10,000	8.13×10^5	6.8 min	>1 h
Isolated***	10,000	3.24×10^4	0.3 min	<10 min

* Flow rate: 2,000 events/s
** Considering 9 samples (3 experimental groups × 3 replicas/group). Includes 45 s automated mixing and rinsing between samples on the MACSQuant® Analyzer
*** Isolation using CD8 (TIL), CD4 (TIL), or CD4/CD8 (TIL) MicroBeads, respectively

Table 3: Cell enrichment helps to decrease time for flow sorting and analysis. Isolation of CD4⁺, CD8⁺, and pan T cells from different mouse tumor models using mouse CD4 (TIL) MicroBeads, CD8 (TIL) MicroBeads, and CD4/CD8 (TIL) MicroBeads dramatically decreases time of analysis.

Quality control

Quality control of samples prior to downstream genomic analysis can save time, costs, and set the right expectation for the outcome of the analysis. Especially when performing single-cell genomic analyses, there are certain requirements a sample must meet to achieve reliable, reproducible, and accurate results. These requirements include having cell viability above 90%, being in a single-cell suspension, and having an accurate cell count. The MACSQuant® Analyzer Flow Cytometers allow you to check all these parameters at once.

The MACSQuant Analyzers make flow cytometry easy for any user. With their unique function of volumetric pipetting, absolute cell counting is automatically provided with every sample measurement and every population being analyzed without the need for counting beads and in less than a minute. As little as 25 µL of your sample is enough to obtain an accurate total cell count and determine the viability of your cells of interest. Furthermore, the Count and Live Cell Discrimination Express Modes enable you to obtain the results of the number of viable cells present in the sample automatically.

There are many other innovative and beneficial features of the MACSQuant Analyzers. For example, the autolabeling function for full automation of sample acquisition and the statistic reports available with the integrated software package.

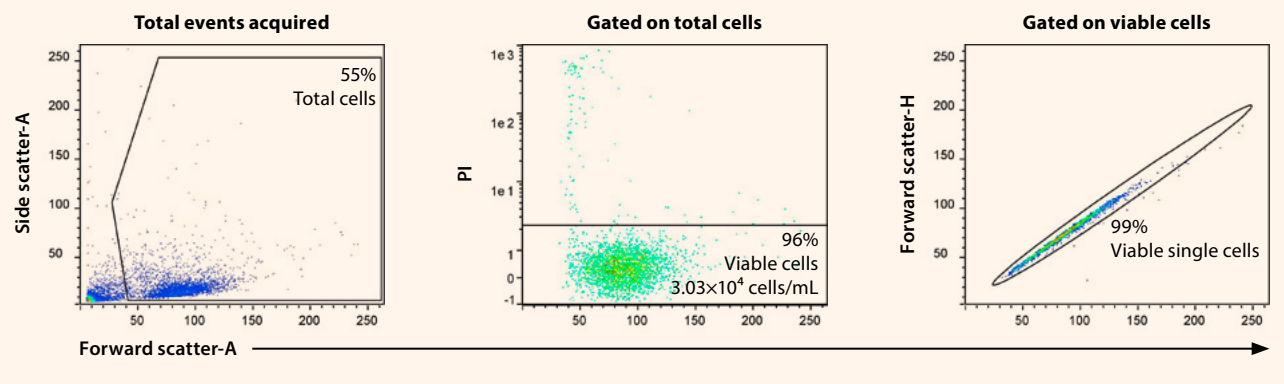


Figure 16: Easy and fast assessment of cell count and cell viability before downstream genomic applications. Exemplary quality control analysis of a neural cell suspension obtained after dissociation of mouse brain and analyzed using a MACSQuant Analyzer 10. Cell viability was determined using the DNA intercalating dye propidium iodide (PI) and the autolabeling function of the instrument.

Tips and information

Our recommendations for the preparation of cells and nuclei for genomic analyses.

Best practice tips

- Prepare your samples as fast as possible. Minimize cell preparation steps to avoid cell death and changes in the gene expression pattern.
- When possible, use wide-bore pipettes to minimize cell damage and use nuclease-free reagents and consumables.
- Some cells tend to aggregate after tissue dissociation due to free floating DNA. An additional DNAse wash after dissociation might help reduce cell aggregates.
- Before starting your single-cell analysis, obtain the total cell count of your final suspension and analyze cell viability.
- Remove dead cells when viability is below 90% to increase the recovery of viable cells.
- Increase the recovery of nucleated cells by removing red blood cells. However, red blood cell lysis may induce cell stress responses. Therefore, apply red blood cell lysis only if necessary. For example, if target cells are isolated, removal of red blood cells may be dispensable.
- Remove debris and cell clumps from your sample before analysis. This allows for more accurate cell counts and avoids clogging during cell sorting, as well as on the single-cell genomics platform.
- Apply cell or nuclei suspensions to your single-cell genomics platform immediately after preparation. Storage may lead to clumping of cells or nuclei. If storage is unavoidable, store samples on ice for a short period of time, not exceeding 30 minutes.

Product information

Product	Order no.
Instruments	
gentleMACS Octo Dissociator with Heaters	130-096-427
autoMACS Pro Separator Starter Kit	130-092-545
MACSQuant Tyto Cell Sorter	130-103-931
For more information about the MACSQuant Analyzers Flow Cytometers visit **miltenyibiotec.com/macsquant4genomics**	
Reagents	
MACS Tissue Storage Solution	130-100-008
Dead Cell Removal Kit	130-090-101
Debris Removal Solution	130-109-398
Red Blood Cell Lysis Solution (10x)	130-094-183
Nuclei Extraction Buffer	130-128-024
For a complete list of Tissue Dissociation Kits, visit **miltenyibiotec.com/TDK4genomics**	
For a complete list of MicroBeads and Isolation kits, visit **miltenyibiotec.com/kits4genomics**	
For a complete list of StraightFrom products visit **miltenyibiotec.com/SF4genomics**	
For more information about products for direct isolation of PBMCs from blood **contact us**	

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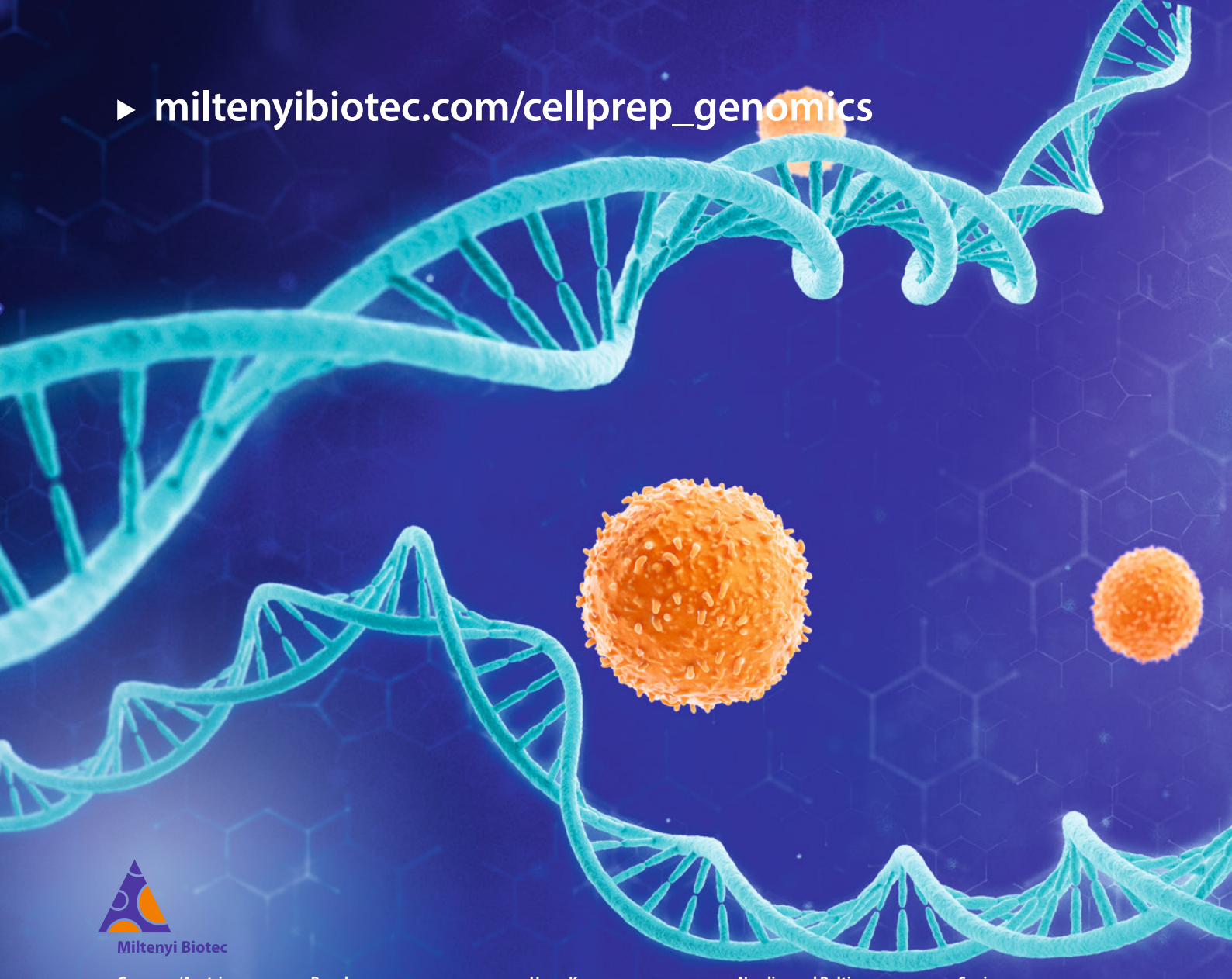
▸ **miltenyibiotec.com/data2download**

▶ miltenyibiotec.com/cellprep_genomics



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