gentleMACS 儀器教學

產品專員: 許育禎
日期:2021/02/04

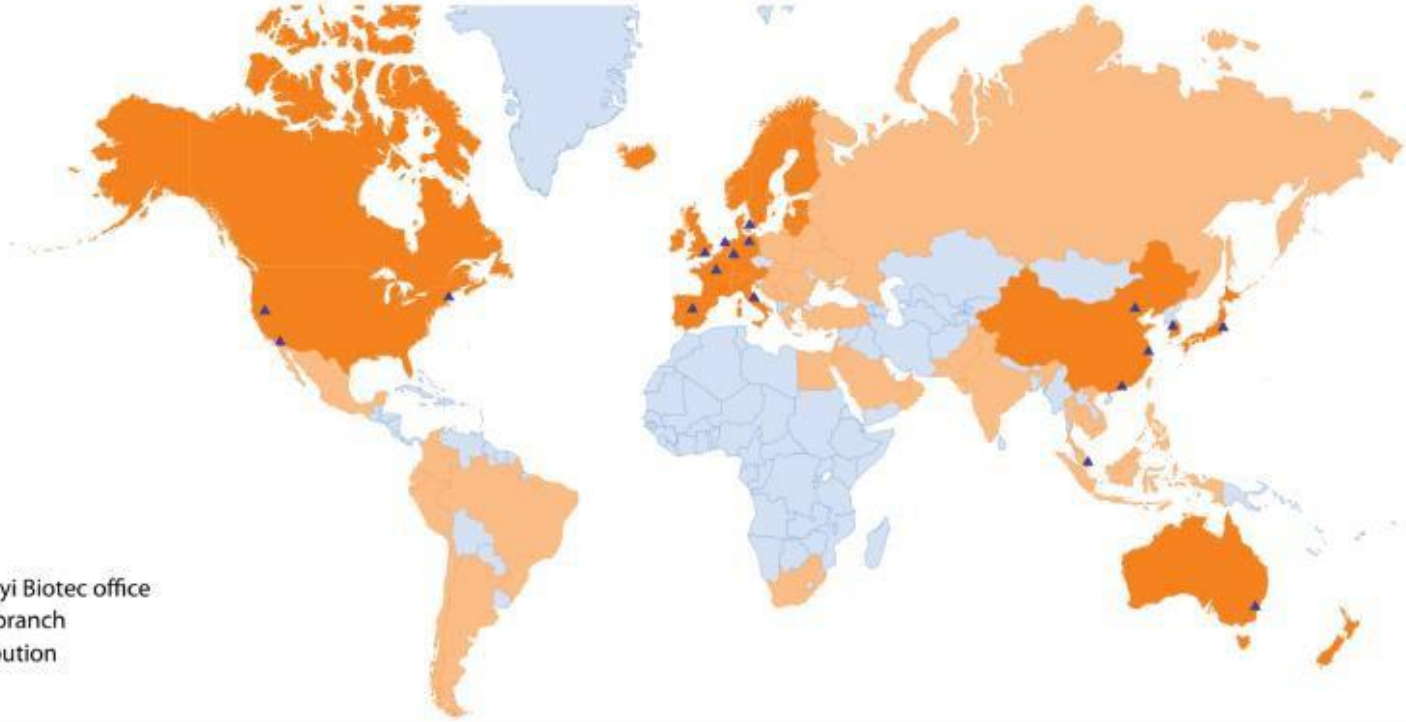
元昕實業有限公司

Global presence

Direct presence in 25 countries
Distribution to 34 additional countries
Two major sites in Germany
Bergisch Gladbach, Headquarters
Teterow, GMP manufacturing facility





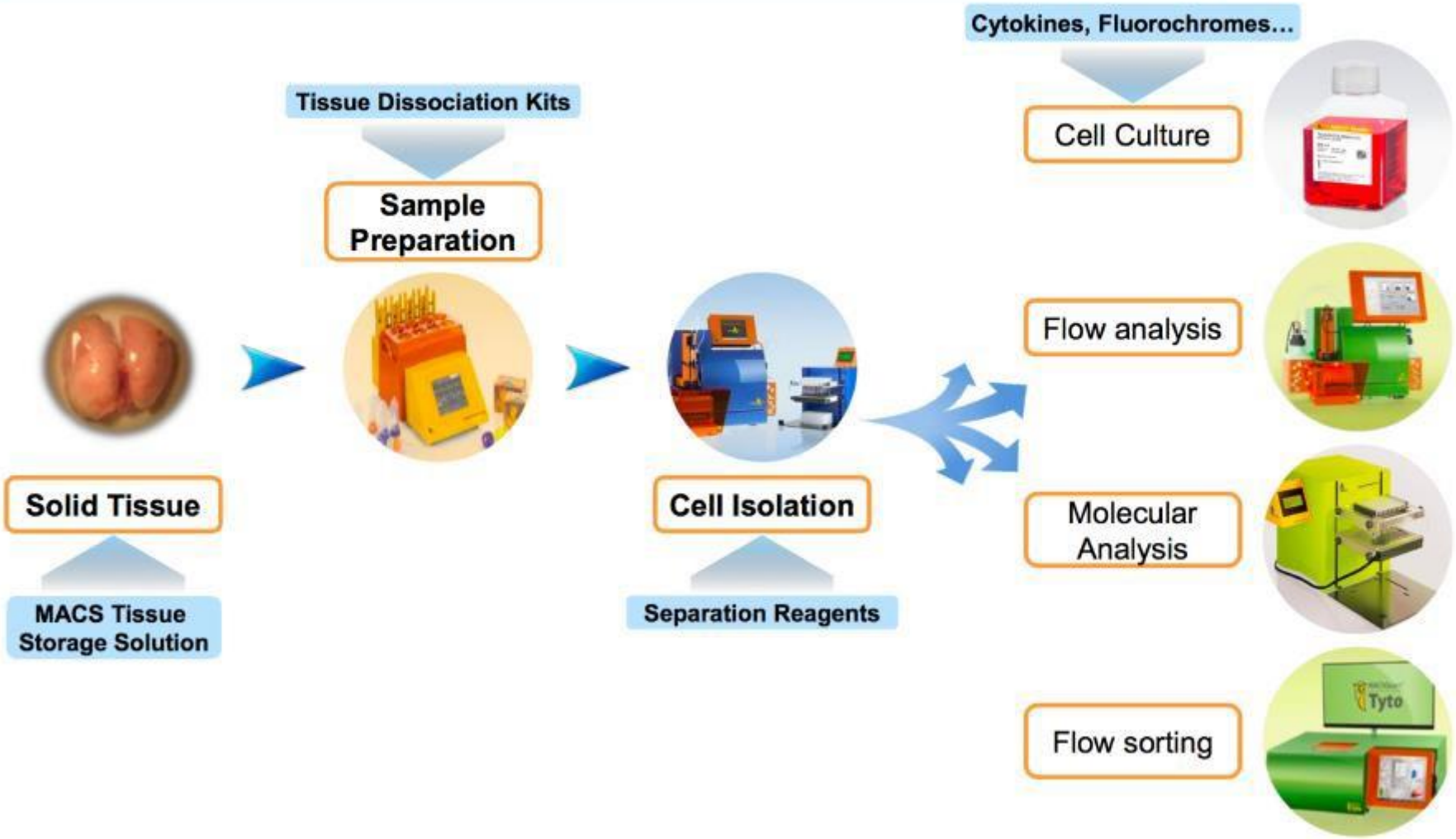


Product portfolio- from benchtop to bedside



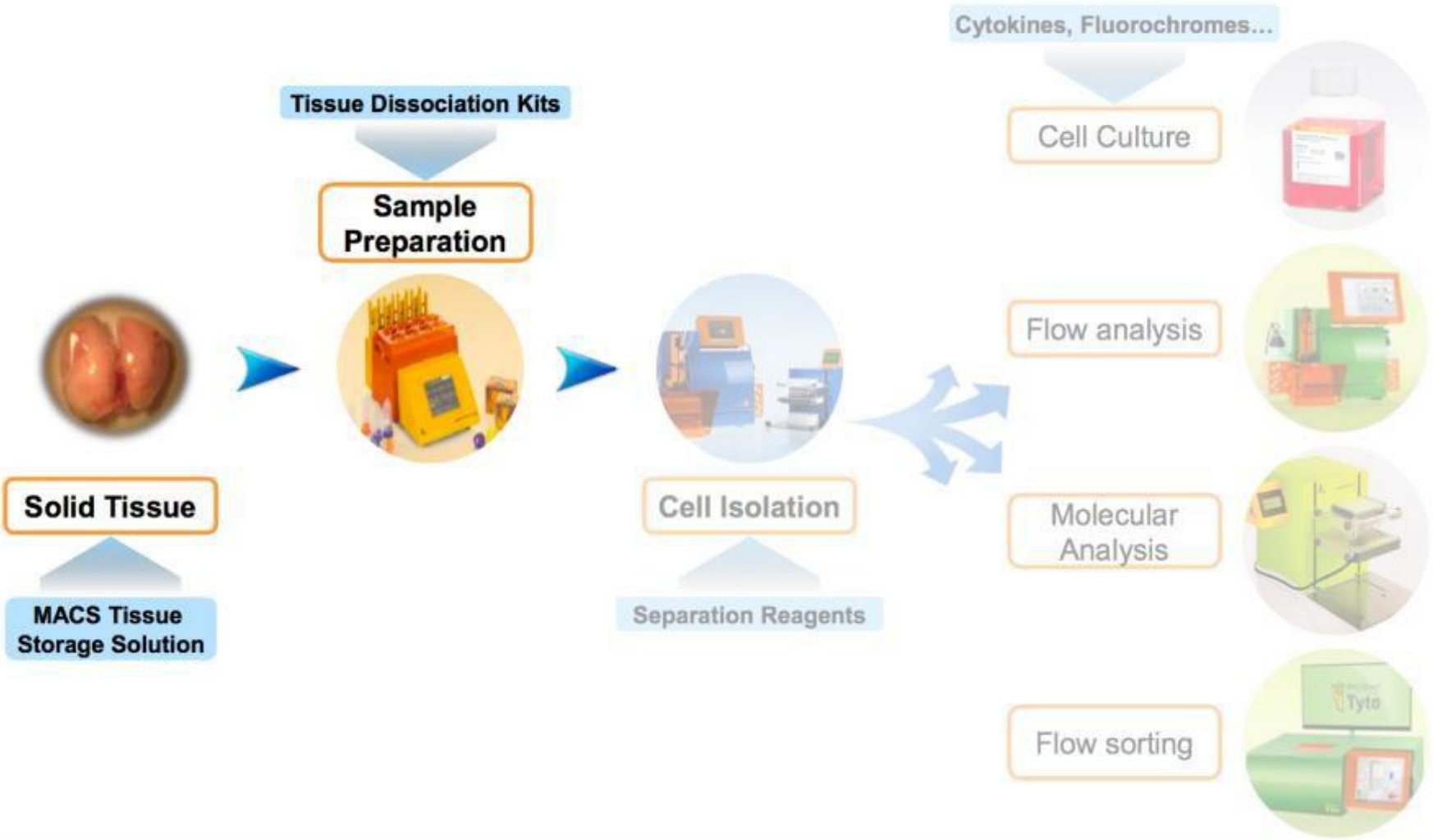
Your Research workflow

MACS
Miltenyi Biotec



Your Research workflow

Miltenyi Biotec



gentleMACS Technology

gentleMACS™ Technology – Tissue dissociation



Mechanical disruption



Enzymatic digestion



gentleMACS™ 組織處理器

			
處理通量	8	8	2
單細胞製備	√	√	√
加熱模組	√	-	-
自動化程度	全自動	半自動	半自動
免費程式升級	√	√	√

gentleMACS C Tubes

to obtain single-cell suspensions





gentleMACS M Tubes

automated tissue homogenization



single-cell suspensions for cell separation, cell analysis, and cell culture.



in order to isolate biomolecules like mRNA or total RNA, or to extract proteins





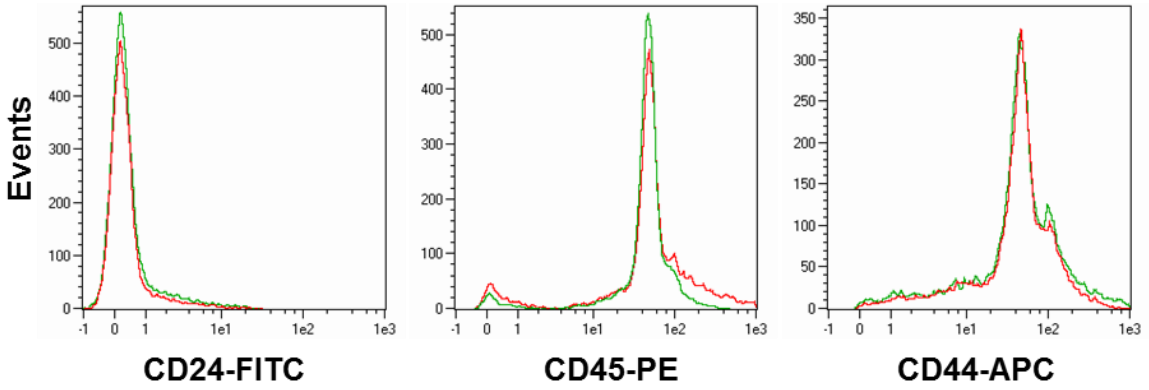
MACS® Tissue Dissociation Kits

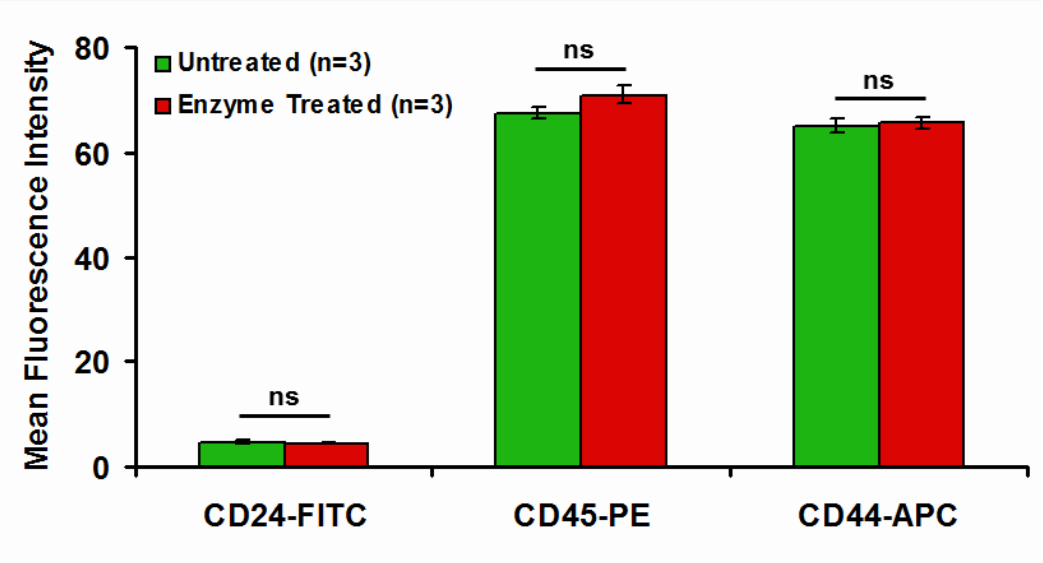
- Gentle: Preserves membrane integrity and surface epitopes
- Fast: Get quality single-cell suspensions in less time
- Reproducible: Standardized procedures for user-independent results



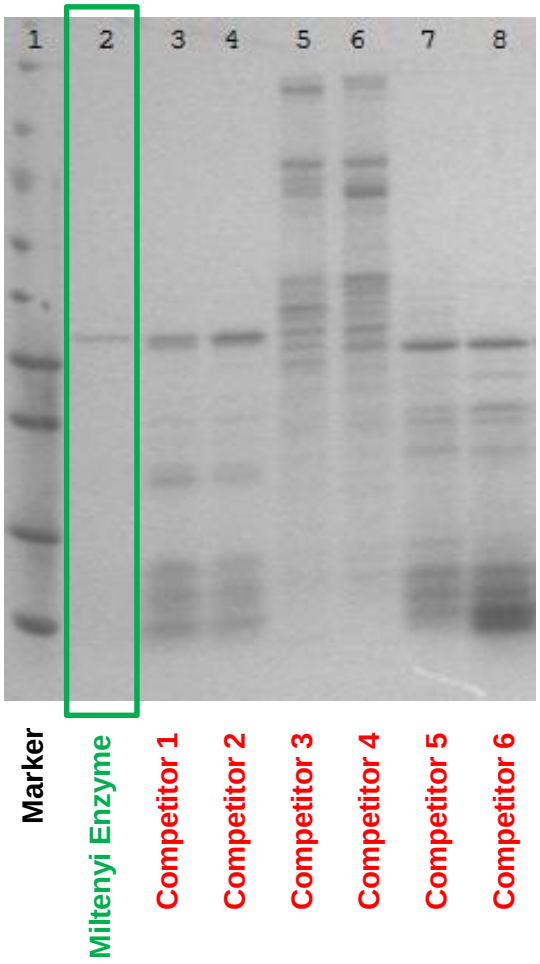
Order no.	Product	Number of reactions	Order no.	Product	Number of reactions
130-095-929	Tumor Dissociation Kit, human	25	130-105-807	Liver Dissociation Kit, mouse	25
130-096-730	Tumor Dissociation Kit, mouse	50	130-095-927	Lung Dissociation Kit, mouse	50
130-105-737	Umbilical Cord Dissociation Kit, human	25	130-097-340	Mitochondria Extraction Kit – Tissue	25
130-101-540	Whole Skin Dissociation Kit, human	50	130-098-373	Neonatal Heart Dissociation Kit, mouse and rat	25
130-105-808	Adipose Tissue Dissociation Kit, mouse and rat	50	130-092-628	Neural Tissue Dissociation Kit (P)	50
130-107-677	Adult Brain Dissociation Kit, mouse and rat	50	130-093-231	Neural Tissue Dissociation Kit (T)	50
130-095-942	Brain Tumor Dissociation Kit (P), human	25	130-094-802	Neural Tissue Dissociation Kit – Postnatal Neurons	50
130-095-939	Brain Tumor Dissociation Kit (T), human	25	130-095-943	Neurospheres Dissociation Kit (P)*	100
130-096-348	Embryoid Body Dissociation Kit, human and mouse	50	130-095-944	Neurospheres Dissociation Kit (T)*	100
130-103-464	Epidermis Dissociation Kit, human	100	130-098-305	Skeletal Muscle Dissociation Kit, mouse and rat	50
130-095-928	Epidermis Dissociation Kit, mouse	25	130-095-926	Spleen Dissociation Kit, mouse	50
130-097-410	Lamina Propria Dissociation Kit, mouse	50	130-118-052	FFPE Tissue Dissociation Kit	25

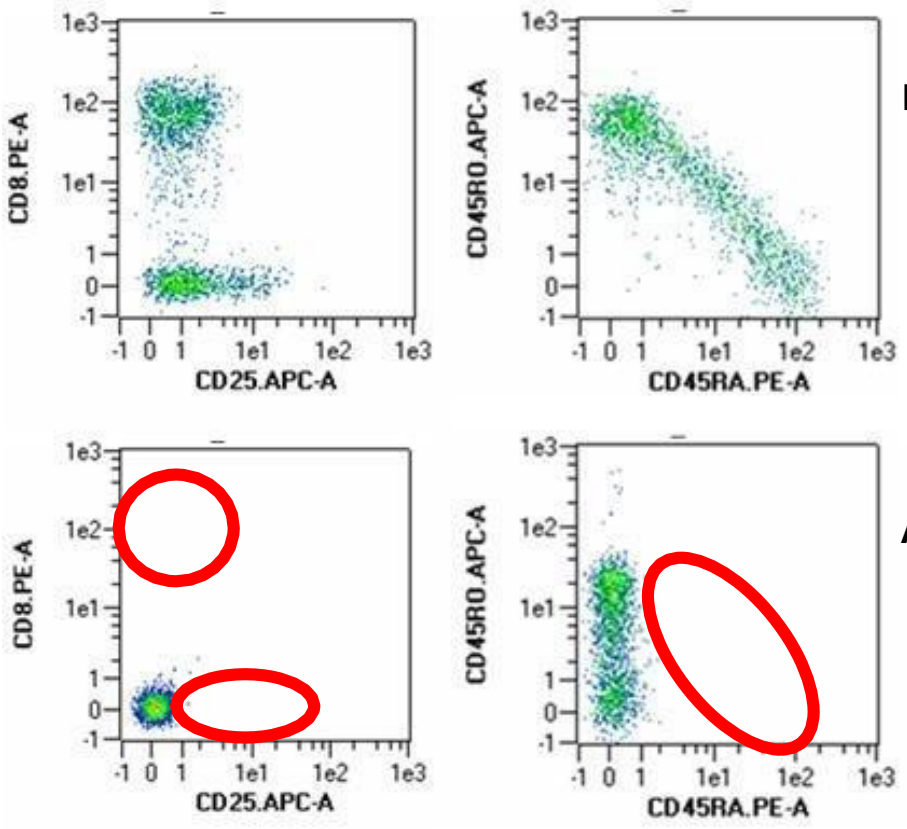
optimized enzyme mixes





Preserve cell integrity and surface epitopes: MB Dissociation Kit vs.Crude Enzymes





MB dissociation kit (highly purified)

Alternative enzyme combination (crude)

Degradation of CD8,CD25, CD45RA, TCRγδ epitopes (human tumor)

Preservation of cell surface epitopes

Tumor Dissociation Kit, human

Preservation of cell surface epitopes

Background

The Tumor Dissociation Kit, human enables the gentle dissociation of tumor tissue, resulting in viable, functional single cells. The dissociation process involves enzymatic digestion steps. Here we tested whether the three enzymes used for enzymatic digestion affect the integrity of cell surface epitopes on the treated cells. Our results show that the vast majority of more than 200 epitopes tested is well preserved during enzymatic treatment.

Method

Tumor cell lines mixed with PBMCs (ratio 1:1) were either treated with the Tumor Dissociation Kit enzymes or incubated in buffer without enzymes (2×30 min at 37 °C). The cells that were incubated without enzymes were stained with a cell trace dye. The enzymatically treated and untreated samples were then mixed and stained with each of the fluorochrome-conjugated MACS® Antibodies detecting cell surface epitopes and analyzed by flow cytometry. The cell trace dye enabled the distinction between treated and untreated cells. A decrease in fluorescence signal intensity of the fluorochrome conjugates in the treated cells indicated effects on epitope integrity.

Results

Antibody detecting cell surface epitope	Antibody clone	Antibody detecting cell surface epitope	Antibody clone	Antibody detecting cell surface epitope	Antibody clone	Antibody detecting cell surface epitope	Antibody clone
Anti-CLA	HECA-452	Anti-LGR5	DA06-22H28	CD4	VIT4	CD27	M-T271
Anti-CLIP	REA396	Anti-MICA/MICB	6D4	CD5	UCHT2	CD28	15E8
Anti-CXCR1	2A9-1	Anti-MK p80	4A4.D10	CD6	M-T411	CD29	TS2/16
Anti-DCIR	REA329	Anti-Notch2	MHN2-25	CD8	BW135/80	CD31	AC128
Anti-DLL1	MHD1-314	Anti-PTK7 (CCK-4)	188B	CD9	SN4 C3-3A2	CD32	2E1
Anti-DR-3	JD3	Anti-ROR-1	2A2	CD10	97C5	CD33	AC104.3E3
Anti-FcεRIα	CRA1	Anti-SSEA-1	REA321	CD11a (ITGAL)	REA378	CD35	E11
Anti-Fibroblast	REA165	Anti-SSEA-4	REA101	CD11b	MY7D3 3.1.5	CD36	AC106
Anti-FMLP receptor	REA169	Anti-TCR-Vα7.2	REA179	CD11c	MJ4-27G12	CD38	IB6
Anti-Galectin-3	M3/38	Anti-TCR-Vδ1	REA173	CD13	REA263	CD39	MZ18-23C8
Anti-HLA Class I B8	REA145	Anti-TCR-Vδ2	123R3	CD14	TÜK4	CD40	HB14
Anti-HLA Class I B44	REA143	Anti-TCRαβ	BW242/412	CD15	VIMC6	CD41b	REA336
Anti-HLA-ABC	REA230	Anti-TCRγδ	11F2	CD16	VEP13	CD42a	REA209
Anti-HLA-B12	REA138	Anti-TSPAN8	DA03-22H28	CD18	TS1/18	CD43	DF-T1
Anti-HLA-DQ	REA303	CD1a	HI149	CD19	LT19	CD44	DB105
Anti-HLA-DR	AC122	CD1c (BDCA-1)	AD5-8E7	CD20	LT20	CD45	5B1
Anti-HLA-DR, DP, DQ	REA332	CD1d	51.1	CD21	HB5	CD45RA	T6D11
Anti-HLA-E	3D12	CD2	LT2	CD22	REA340	CD45RB	REA119
Anti-Jagged2	MHJ2-523	CD3	BW264/56	CD24	32D12	CD45RO	UCHL1
Anti-KLRG1	REA261	CD4	M-T466	CD25	4E3	CD46	REA312

Tumor Dissociation Kit, mouse

Preservation of cell surface epitopes

Background

The Tumor Dissociation Kit, mouse enables the gentle dissociation of tumor tissue, resulting in viable, functional single cells. The dissociation process involves enzymatic digestion steps. Here we tested whether the three enzymes used for enzymatic digestion affect the integrity of cell surface epitopes on the treated cells. Our results show that the vast majority of more than 200 epitopes tested is well preserved during enzymatic treatment.

Method

Mouse lungs and spleens were non-enzymatically dissociated and mixed with mouse blood and mouse tumor cell lines (4T1, CT26.WT and B16-F10). Subsequently, they were either treated with the Tumor Dissociation Kit enzymes or incubated in buffer without enzymes (40 min at 37 °C). The cells that were incubated without enzymes were stained with a cell trace dye. The enzymatically treated and untreated samples were then mixed and stained with each of the fluorochrome-conjugated MACS® Antibodies detecting cell surface epitopes and analyzed by flow cytometry. The cell trace dye enabled the distinction between treated and untreated cells. A decrease in fluorescence signal intensity of the fluorochrome conjugates in the treated cells indicated effects on epitope integrity.

Results

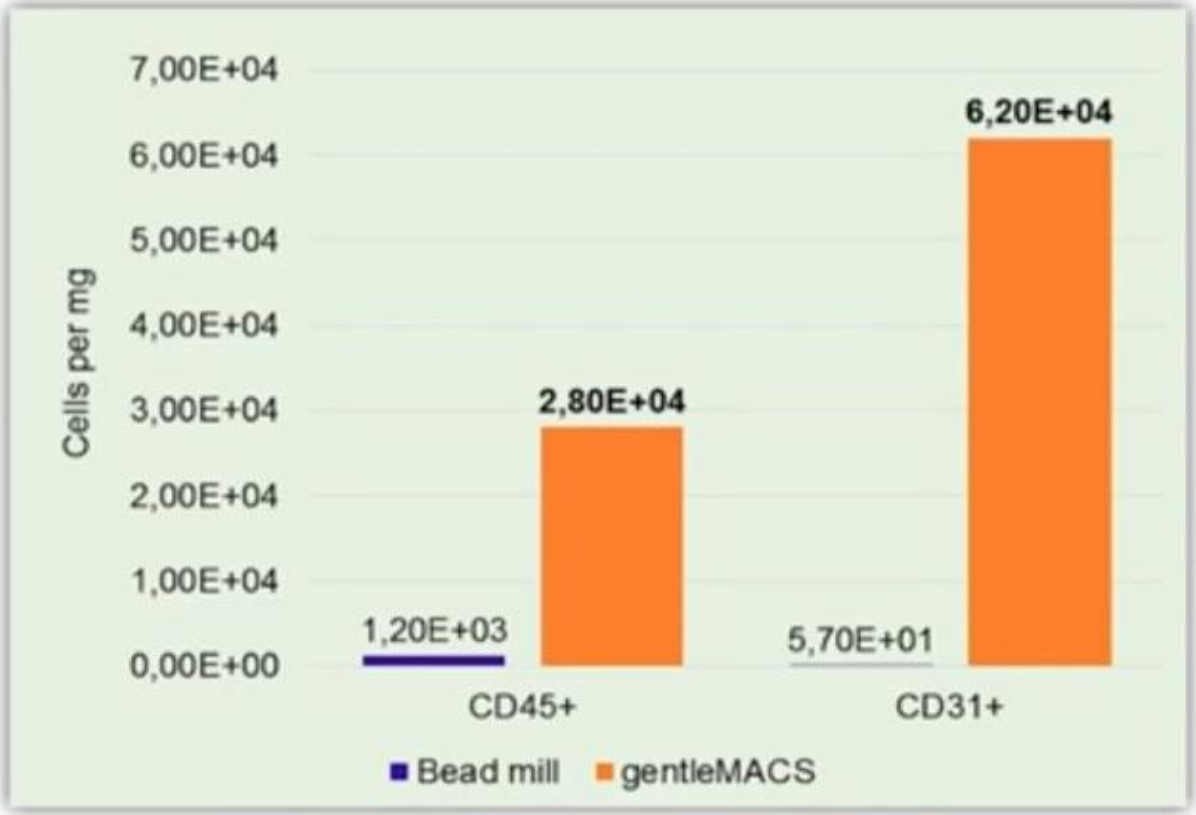
Antibody detecting cell surface epitope	Antibody clone	Antibody detecting cell surface epitope	Antibody clone	Antibody detecting cell surface epitope	Antibody clone	Antibody detecting cell surface epitope	Antibody clone
anti-A2B5	105H829	anti-H60a	REA556	anti-Ly-49	REA241	anti-PSA-NCAM	2-2B
anti-B7-H4	REA392	anti-IgD	11-26c.2a	anti-Ly-49.2	REA115	anti-QA-1B	6A8.6F10.1A6
anti-BP-1	6C3	anti-IgDa	REA494	anti-Ly-6C	1G7.G10	anti-QA-2	REA523
anti-CheckR22	REA451	anti-IgG2ab	X-57	anti-Ly-6G	REA526	anti-Rae-1a/b/g	REA578
anti-DCIR2	33D1	anti-IgM	X-54	anti-MDL-1	REA582	anti-Sca-1	REA422
anti-Dectin-1	REA154	anti-Integrin α7	3C12	anti-Mer	REA477	anti-Siglec F	E50-2440
anti-Endoglin	REA501	anti-Integrin β7	REA441	anti-MHC-II	M5/114.15.2	anti-Siglec H	551.3D3
anti-EphA2	REA579	anti-IFN-γ R β	REA301	anti-MHC-II I-Ab	REA538	anti-Syndecan 4	REA640
anti-F4/80	REA126	anti-Jagged 2	HMJ2-1	anti-MHC-II I-Ak	REA610	anti-TCR δ	REA318
anti-FcεRIa	MAR-1	anti-KLRG1	2F1	anti-MHC-II I-Ek	REA510	anti-TCR γ/δ	GL3
Anti-Feeder Cells	mEF-SK4	anti-LGR5	DA04-10E8.9	anti-Mrl.1	PR136	anti-TCR Vα3.2 (a,c)	REA695
anti-FR-4	TH6	anti-LRAM-1	REA457	anti-Notch1	REA357	anti-TCR Vδ11	REA657
anti-Galectin-3	M3/38	anti-LT-βR	REA476	anti-Notch2	HMN2-35	anti-TCR Vδ14	REA645
anti-GARP	REA129	anti-Ly-49C/F/I/H	14B11	anti-Notch4	HMN4-14	anti-TCR Vδ3.1/δ.2	REA585
anti-GITR (CD357)	DTA-1	anti-Ly-49D	4E5	anti-O4	O4	anti-TCR Vδ4	REA372
anti-GR-1	RB6-8C5	anti-Ly-49E/F	REA738	anti-PIR-A/B	REA472	anti-Ter119	Ter-119
anti-H2Kd/H2Dd	REA527	anti-Ly-49G	AT-8	anti-Plexin B2	REA445	anti-TIGIT	REA536
anti-H2Kk	H100-27A5	anti-Ly-49G2	4D11	anti-Prominin-1	M69-3G8	anti-Tim-3	RMT3-23

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gentleMACS™ Technology – Tissue dissociation

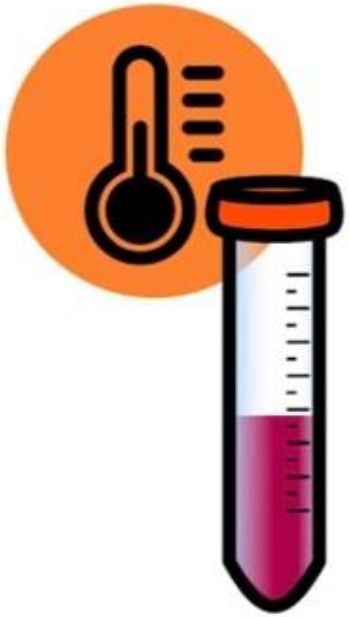


gentleMACS™ vs. bead mill

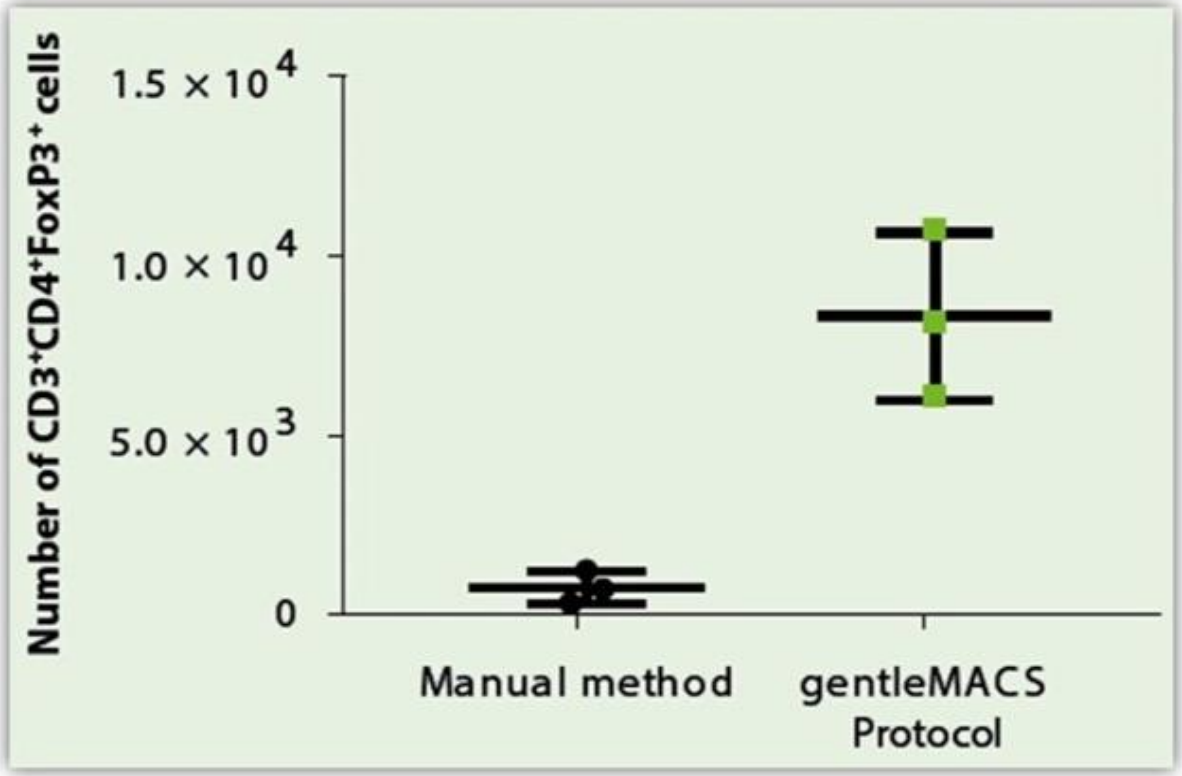


Comparison of numbers of viable CD45+ and CD31+ cells in murine lung, obtained with the gentleMACS™ protocol or following bead mill manufacture´s application note.

gentleMACS™ Technology – Tissue dissociation



gentleMACS™ vs. manual protocol



Comparison of numbers of viable CD3+CD4+FoxP3+ Treg cells in colonic lamina propria, obtained with the gentleMACS Protocol or manual method.

gentleMACS™ Technology



Unique gentleMACS™ Tubes



Easy-to-use Tissue Dissociation Kits

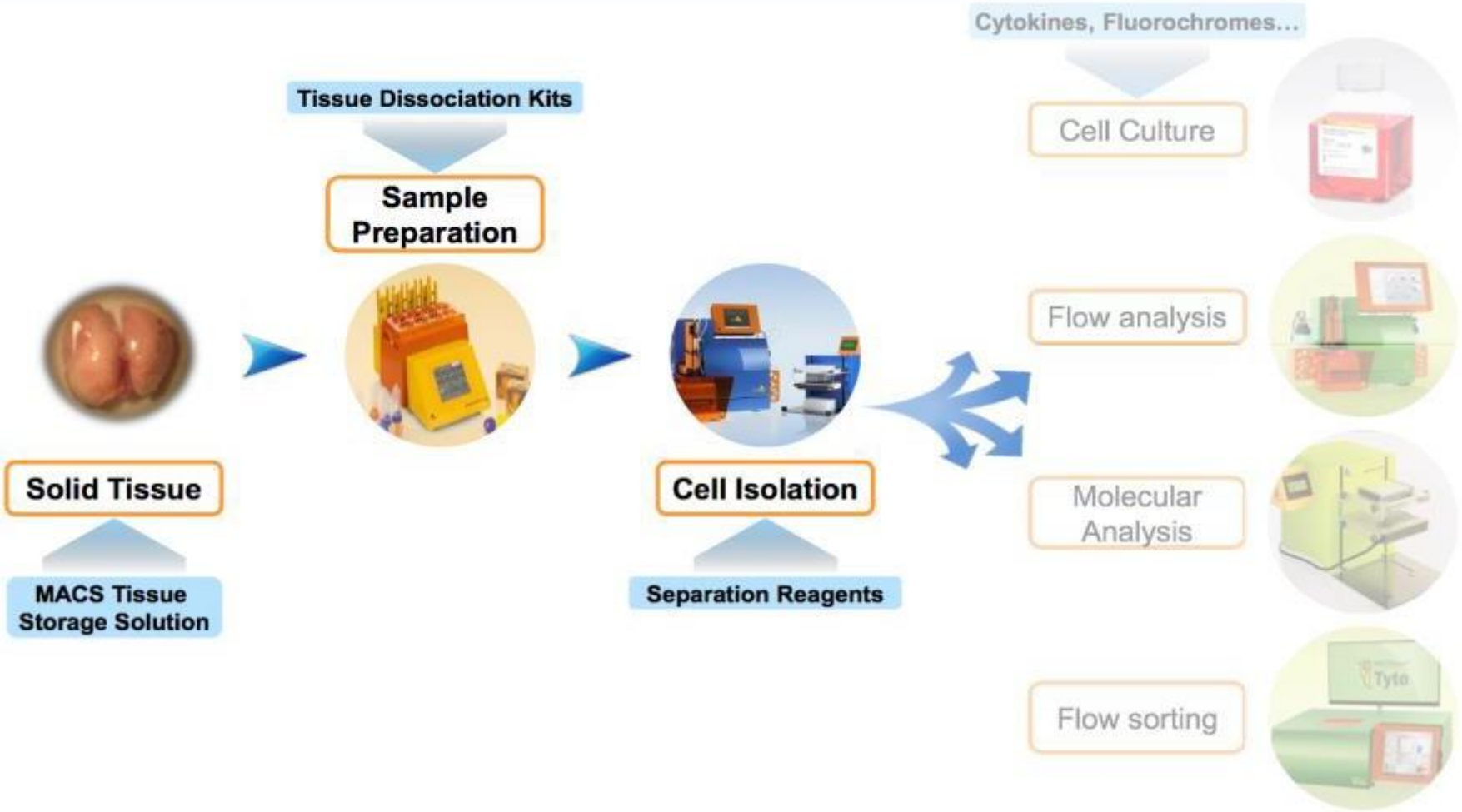
- **fast**
- **gentle**
- **reproducible**



Innovative Instruments:
gentleMACS™ Dissociators

Your Research workflow

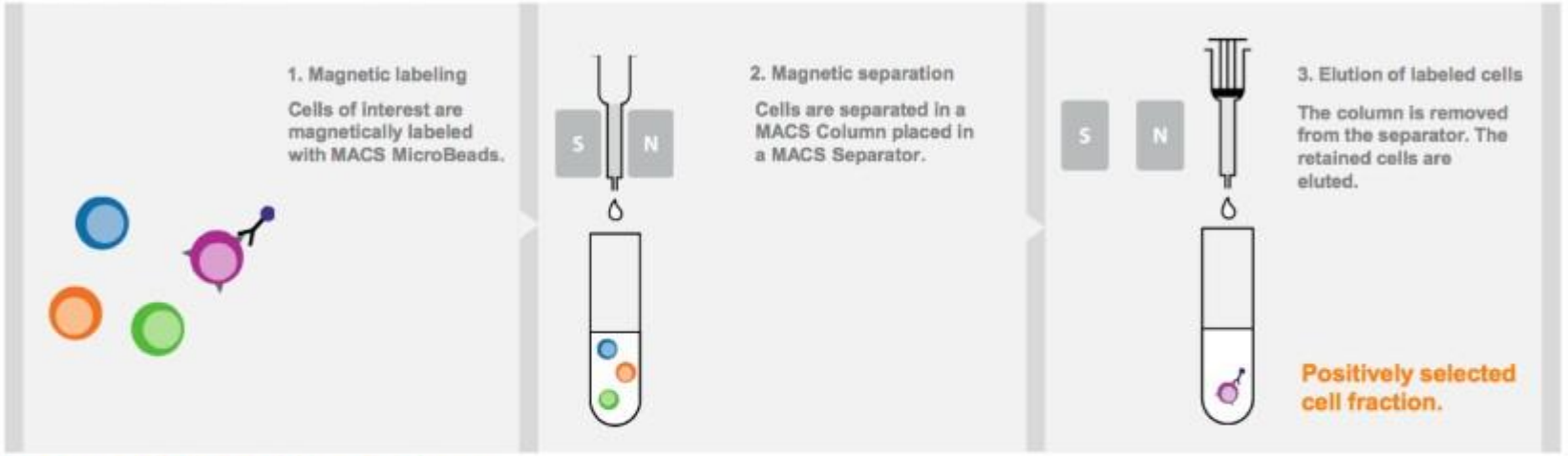
MACS
Miltenyi Biotec



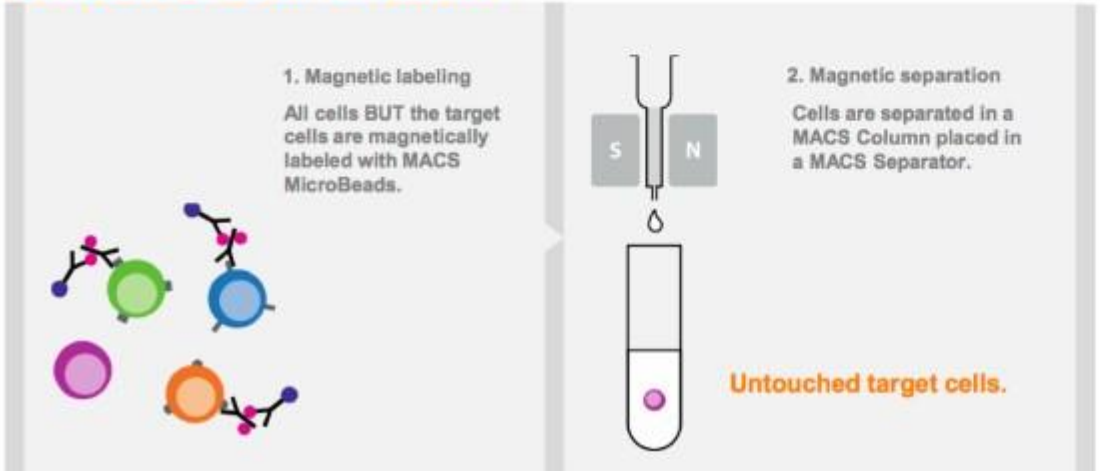
Cell separation with MACS MicroBeads – as easy as 1, 2, 3

Miltenyi Biotec

Positive cell isolation



Untouched cell isolation

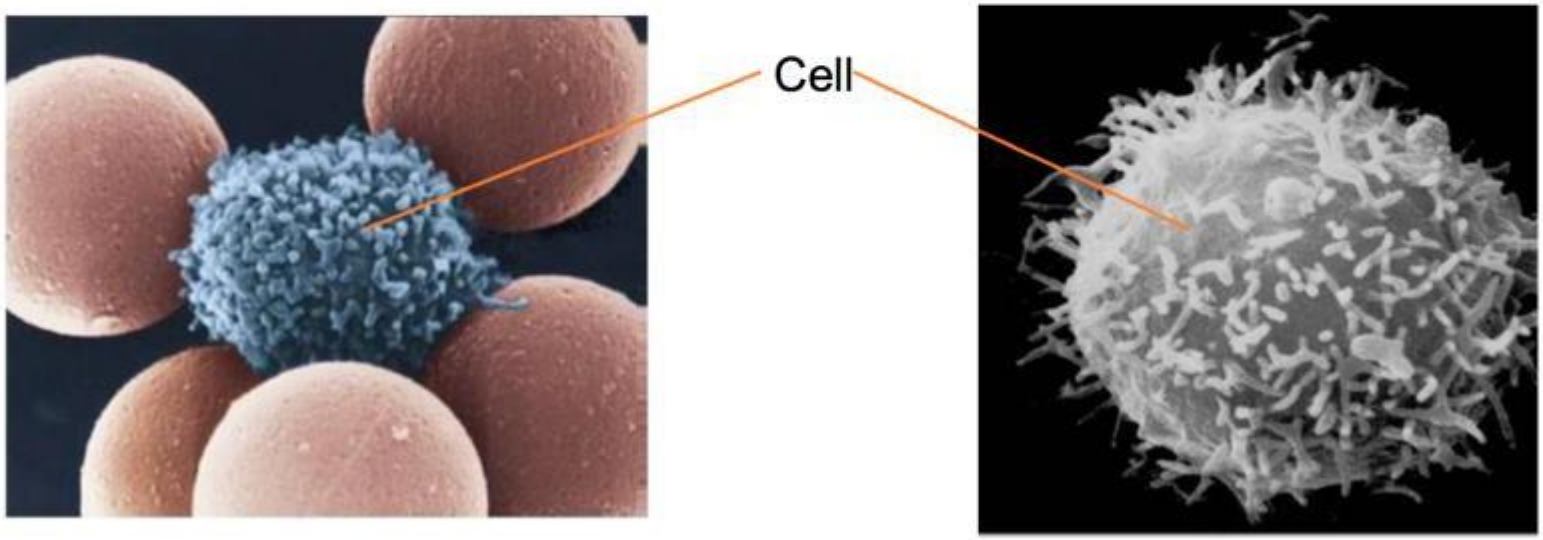


How big are the Beads?

Miltenyi Biotec

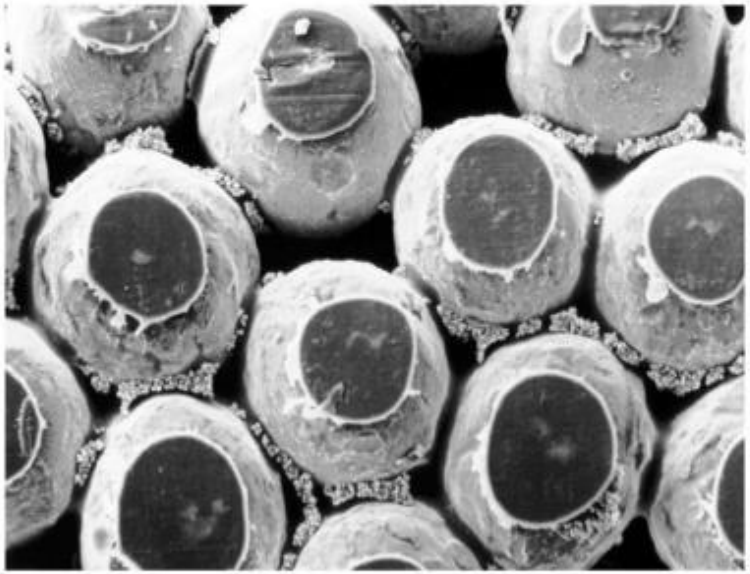
Micro-sized Beads

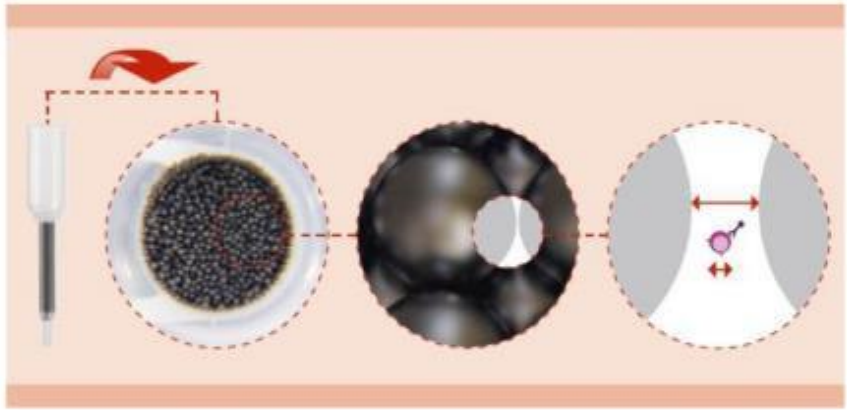
Nano-sized Beads



What to know about MACS® MicroBead Technology?

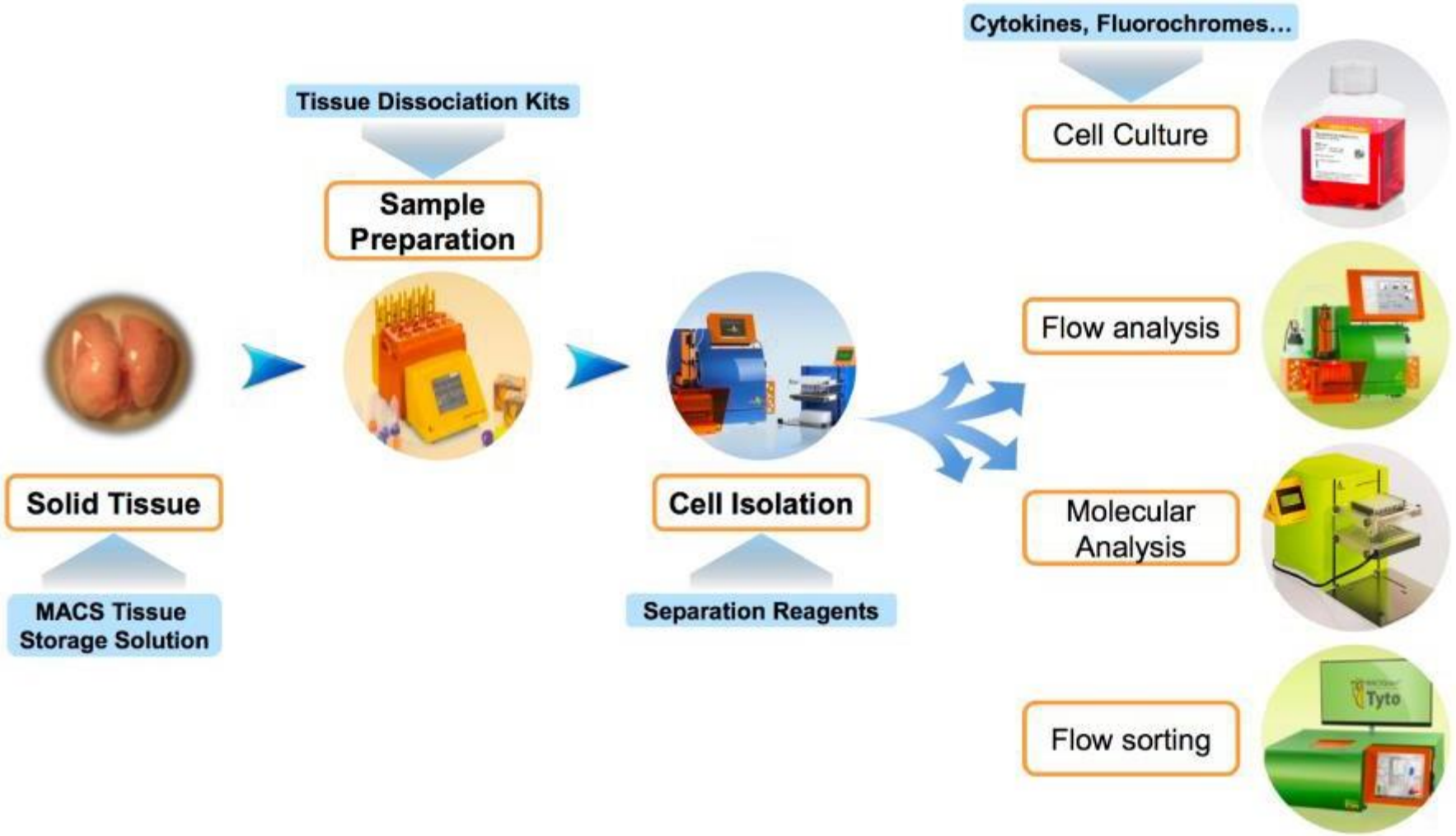
MACS® Columns





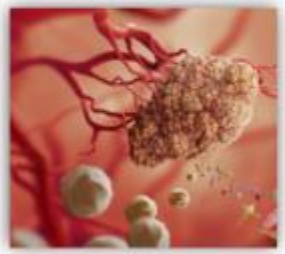
Your Research workflow

Miltenyi Biotec

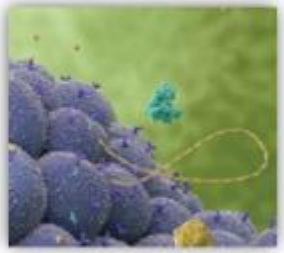


Application Fields

Cancer research
- Tumor dissociation
- and more…



Stem cell research
- Skeletal muscle dissociation
- and more…



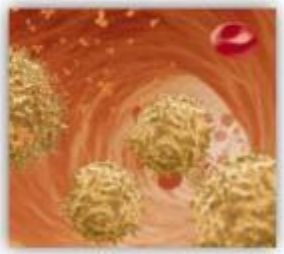
Neuroscience research
- Brain dissociation
- and more…



Cardiovascular research
- Neonatal heart dissociation
- and more…



Immunology research
- Lung dissociation
- Lamina propria dissociation



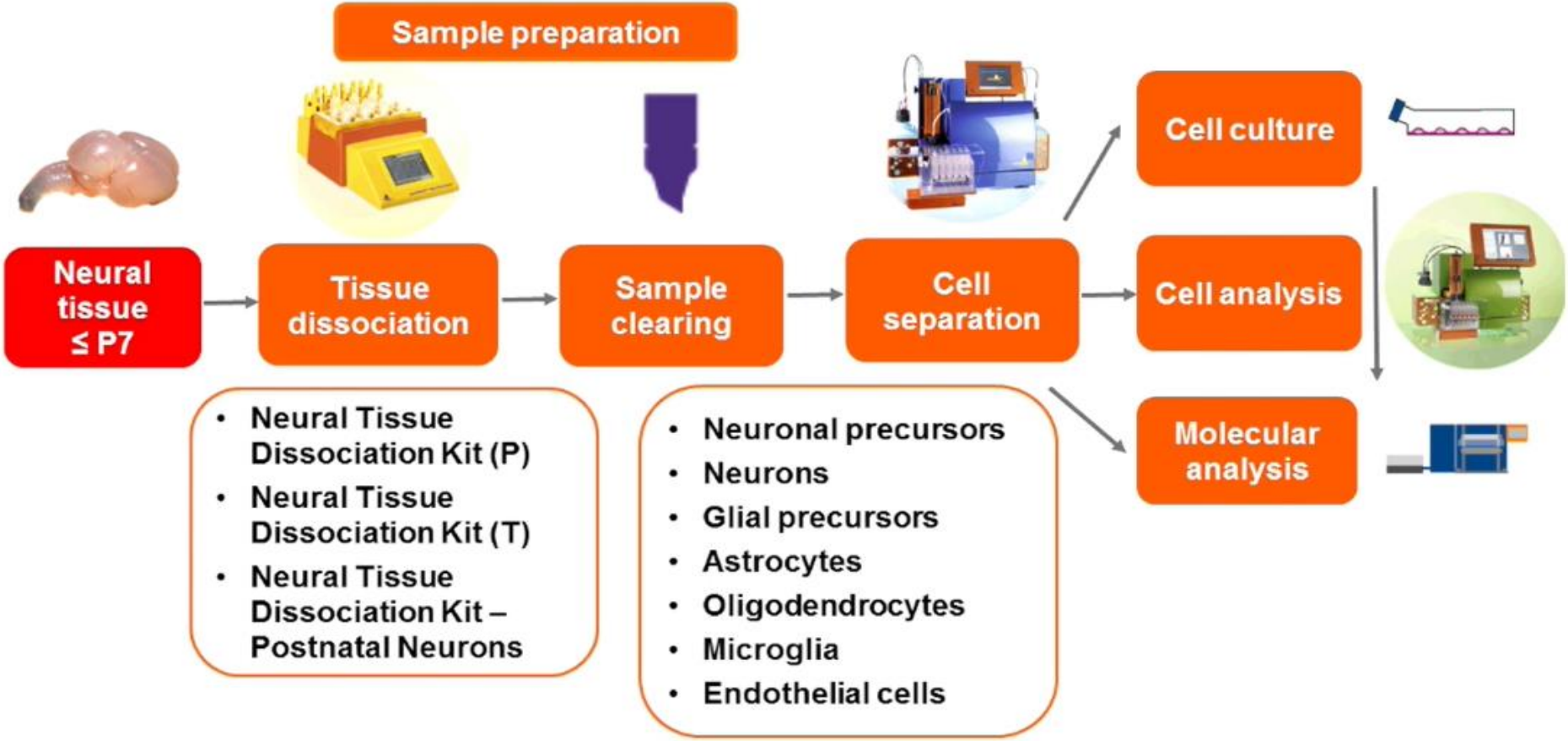


The neonatal brain workflow

MACS
Miltenyi Biotec

Workflow depends on age of animal and cell type of interest:

Animals ≤ P7

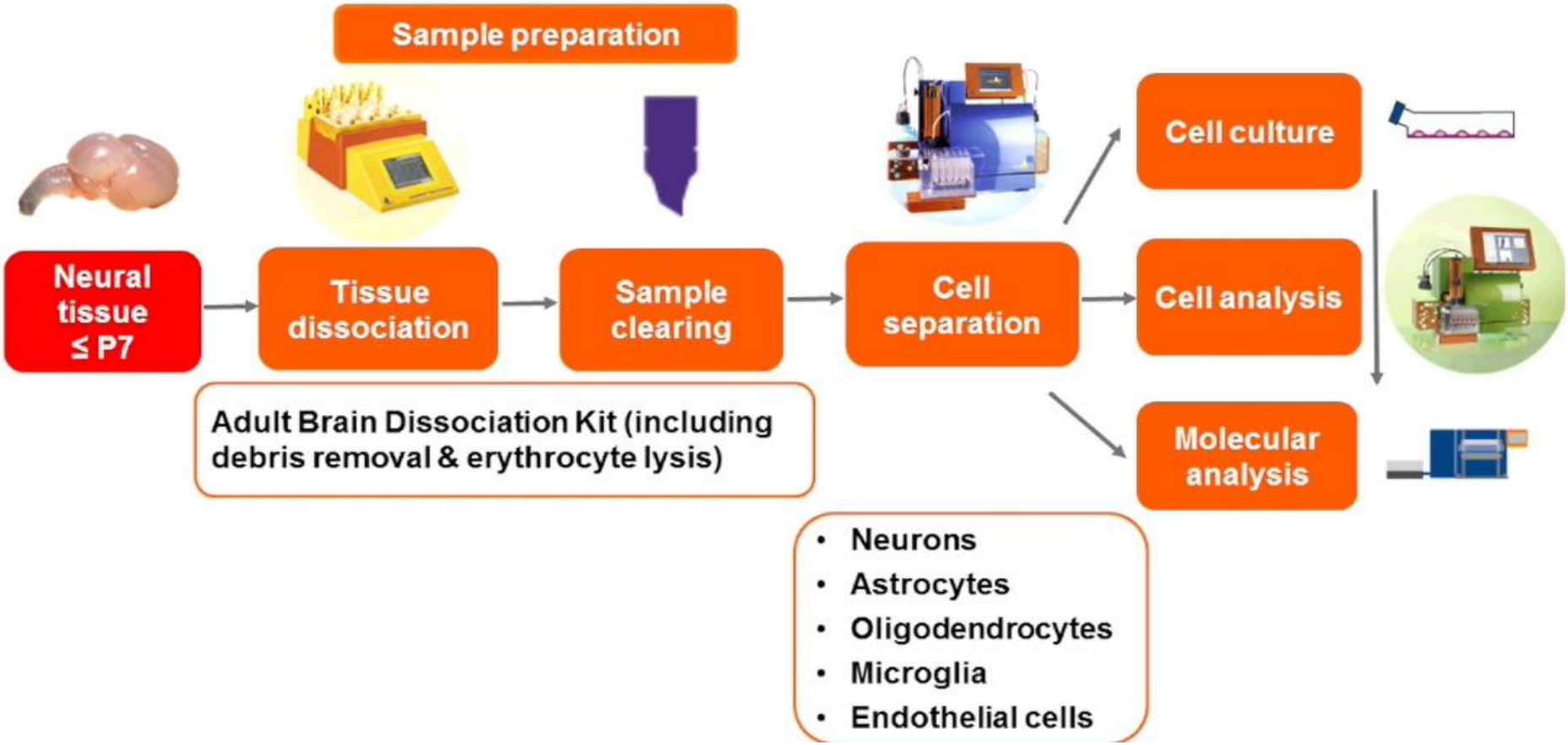


The adult brain workflow

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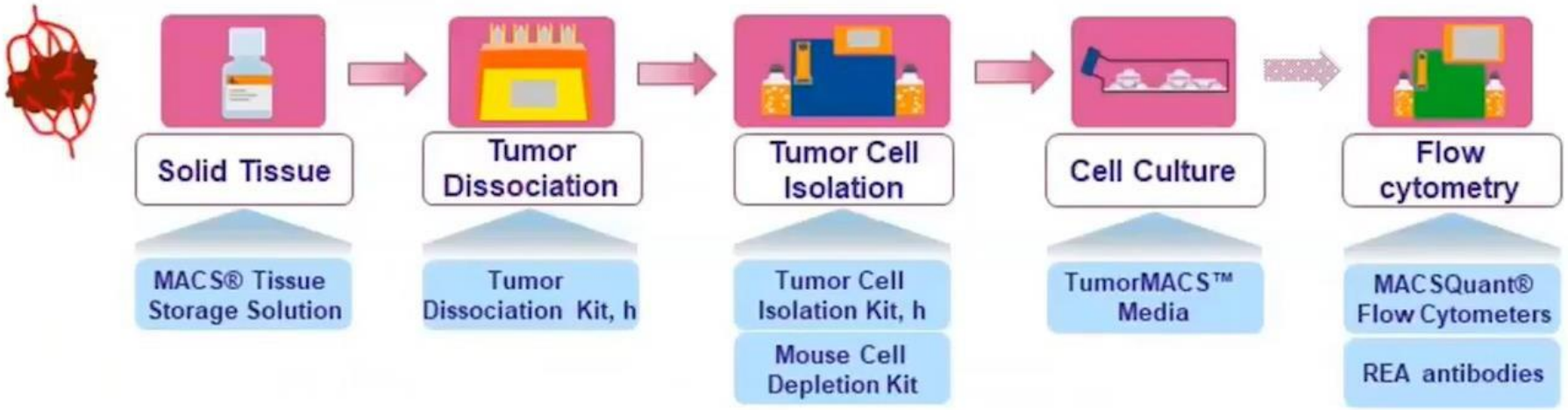
Workflow depends on age of animal and cell type of interest:

Animals > P7



Optimized workflow for primary tumor cell culture

Miltenyi Biotec

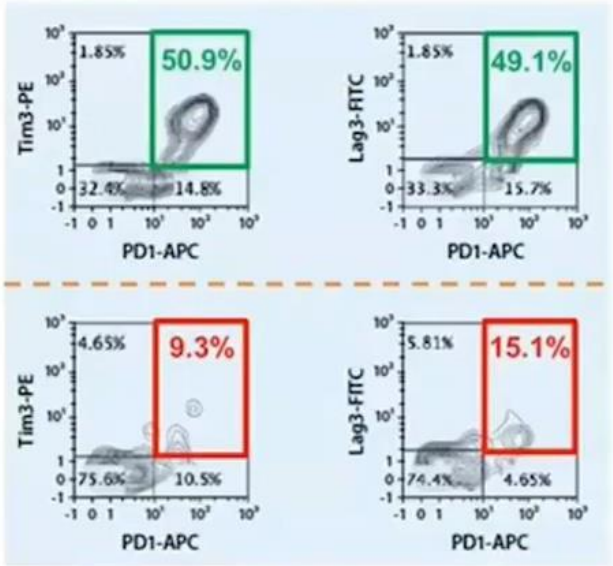


Tumor Dissociation Kit enables the recovery of key TILs

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David Agorku



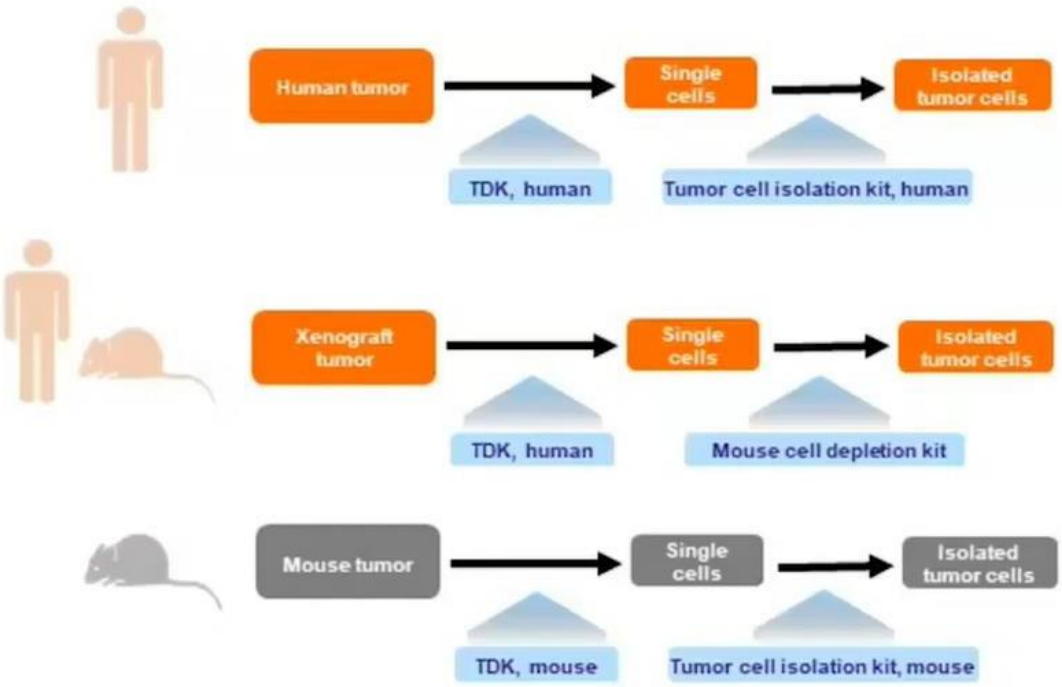
B16-F10 (melanoma) mouse tumors dissociated and labeled with REAfinity antibodies.

From Poster: SITC 2017 Optimized workflow improves the characterization of tumor-infiltrating T cells
https://www.miltenyibiotec.com/upload/assets/IM0019762.PDF

Overview untouched isolation of heterogeneous tumor cells

Miltenyi Biotec

- **Untouched tumor cell isolation by MACS®**



Cancer marker-independent:
- Heterogeneous population of tumor cells isolated
- Suitable for all tumor entities

Beads-free cells:
- Possibility for further beads-based processing (e.g. Dead Cell Removal Kit)
- Possibility for consecutive isolation (e.g. rare cancer stem cells)

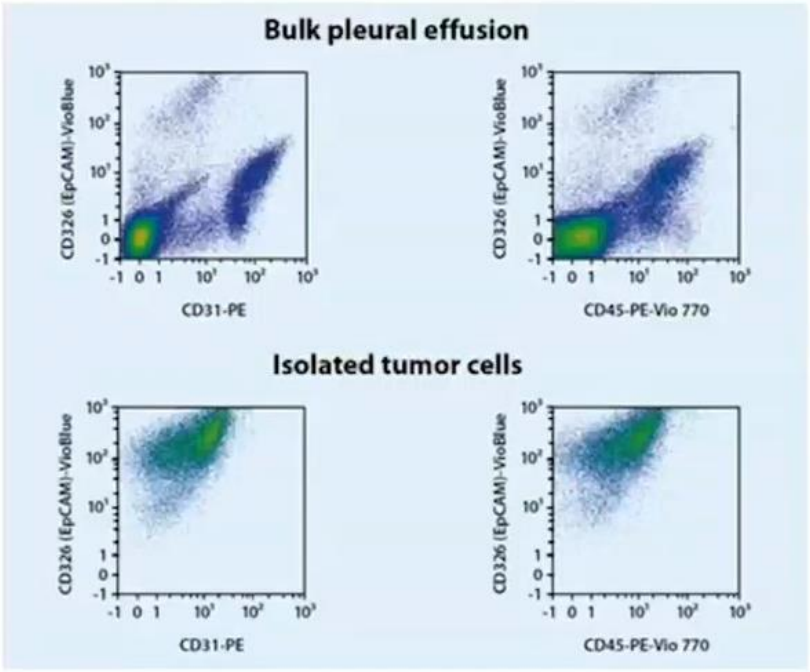
Label-free cells:
- Further deep phenotyping possible

Automation possible on autoMACS® Pro Separator

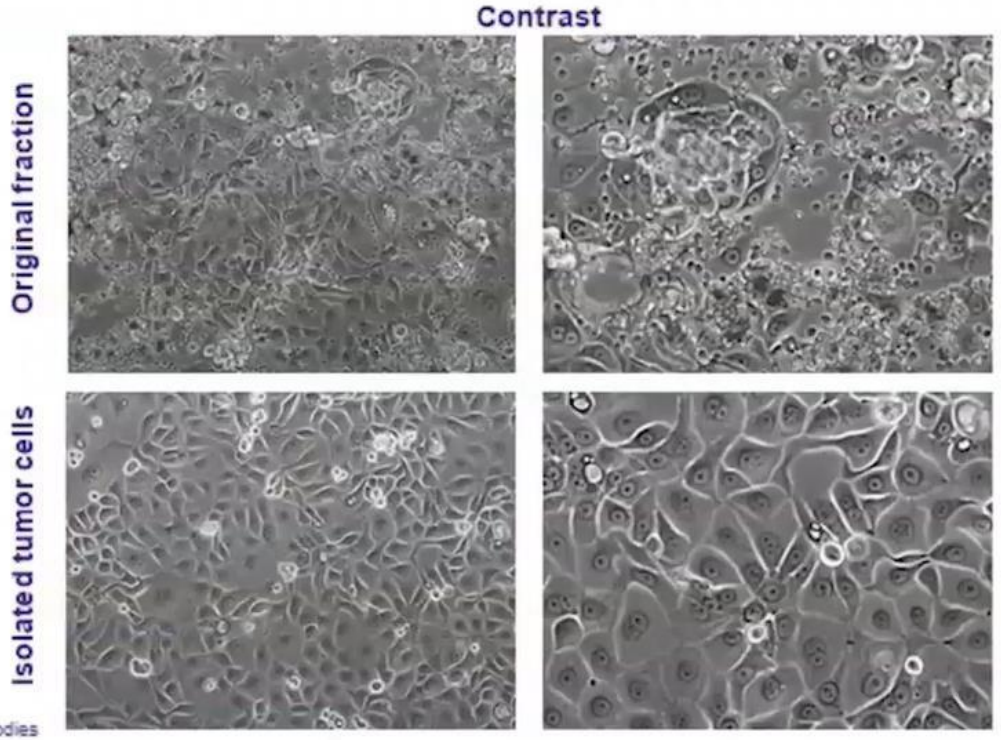
Isolation of human tumor cells from human tumors

Miltenyi Biotec

Tumor Cell Isolation kit, human



Data analyzed with the flow cytometer MACSQuant Analyzer 10 and recombinant REAfinity antibodies

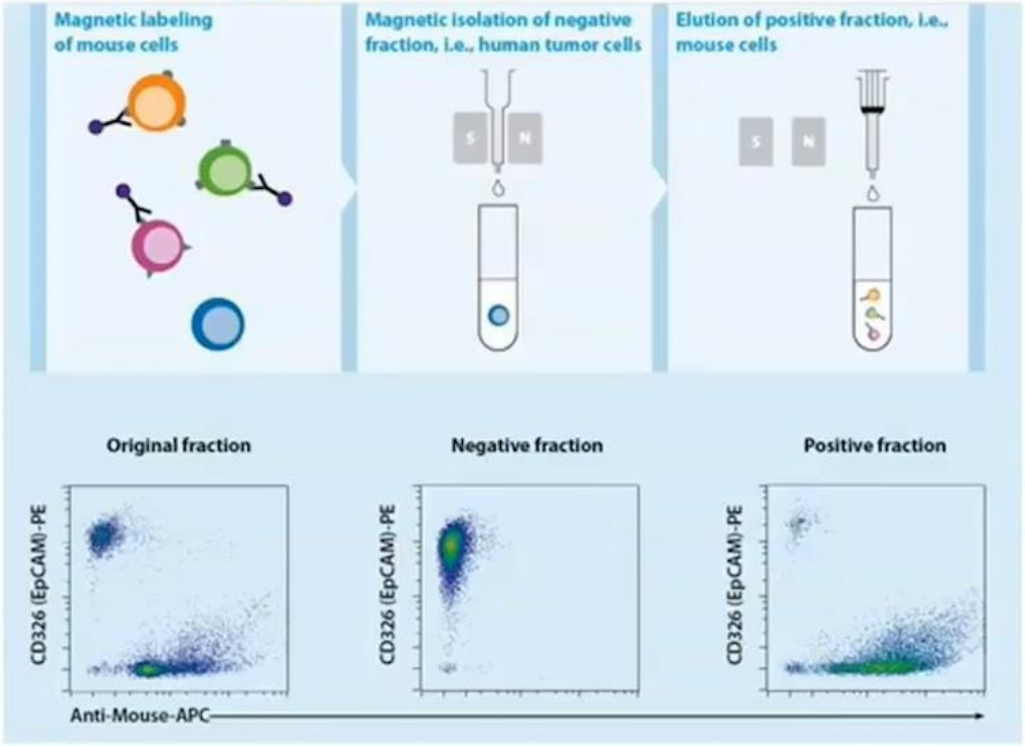


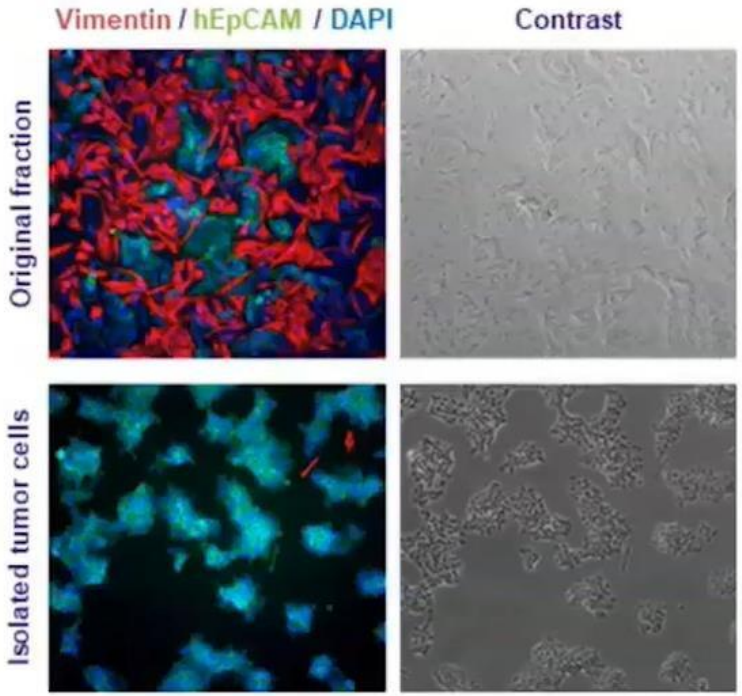
Pleural effusion from ovarian cancer patient

Isolation of human tumor cells from mouse xenografts

Miltenyi Biotec

Mouse Cell Depletion Kit





TumorMACS™ media

Miltenyi Biotec

- **Tumor-entity specific culture media**
  - Serum-free → it does not specifically support the growth of fibroblasts
  - Specifically support growth of tumor cells

- **Efficient *ex vivo* expansion of primary cells**
  - Support plating of cells directly derived from primary or xeno-transplanted tumors
  - Allow for derivation and expansion of stable "primary" cell lines from primary cultures
  - Preserve heterogeneity observed in parental tumor, tumor-initiating capacity and genetic stability
  - Suitable for tumor cells growing in adherent and in suspension cultures
  - 3D setting

- **Improve *in vitro* models for cancer research**

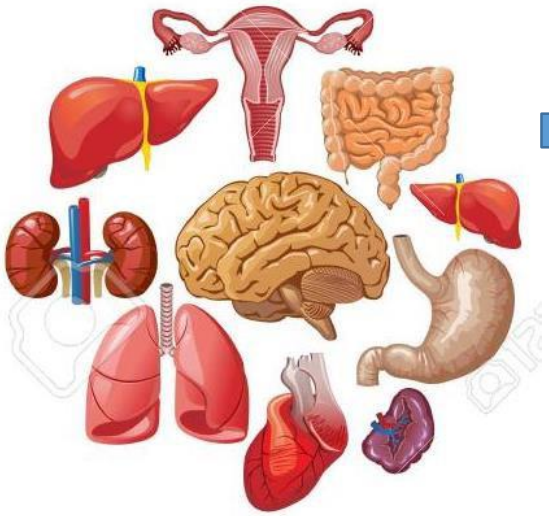


gentleMACS dissociator

Applications



Homogenization of tissue for protein extraction

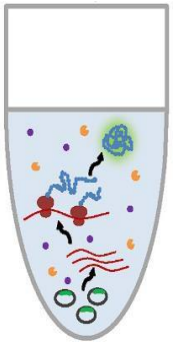


gentleMACS Program
Protein_01.





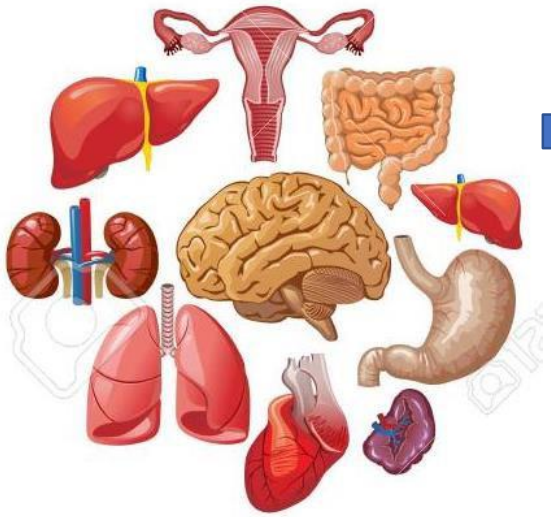
M-tube



廠牌	試劑名稱
Sigma-Aldrich	RIPA Buffer
Sigma-Aldrich	ProteoExtract® Native Membrane Protein Extraction Kit

Homogenization of tissue for total RNA isolation

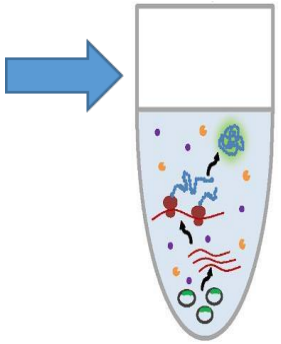
For fresh tissue: gentleMACS Program RNA_01
For frozen tissue: gentleMACS Program RNA_02





M-tube



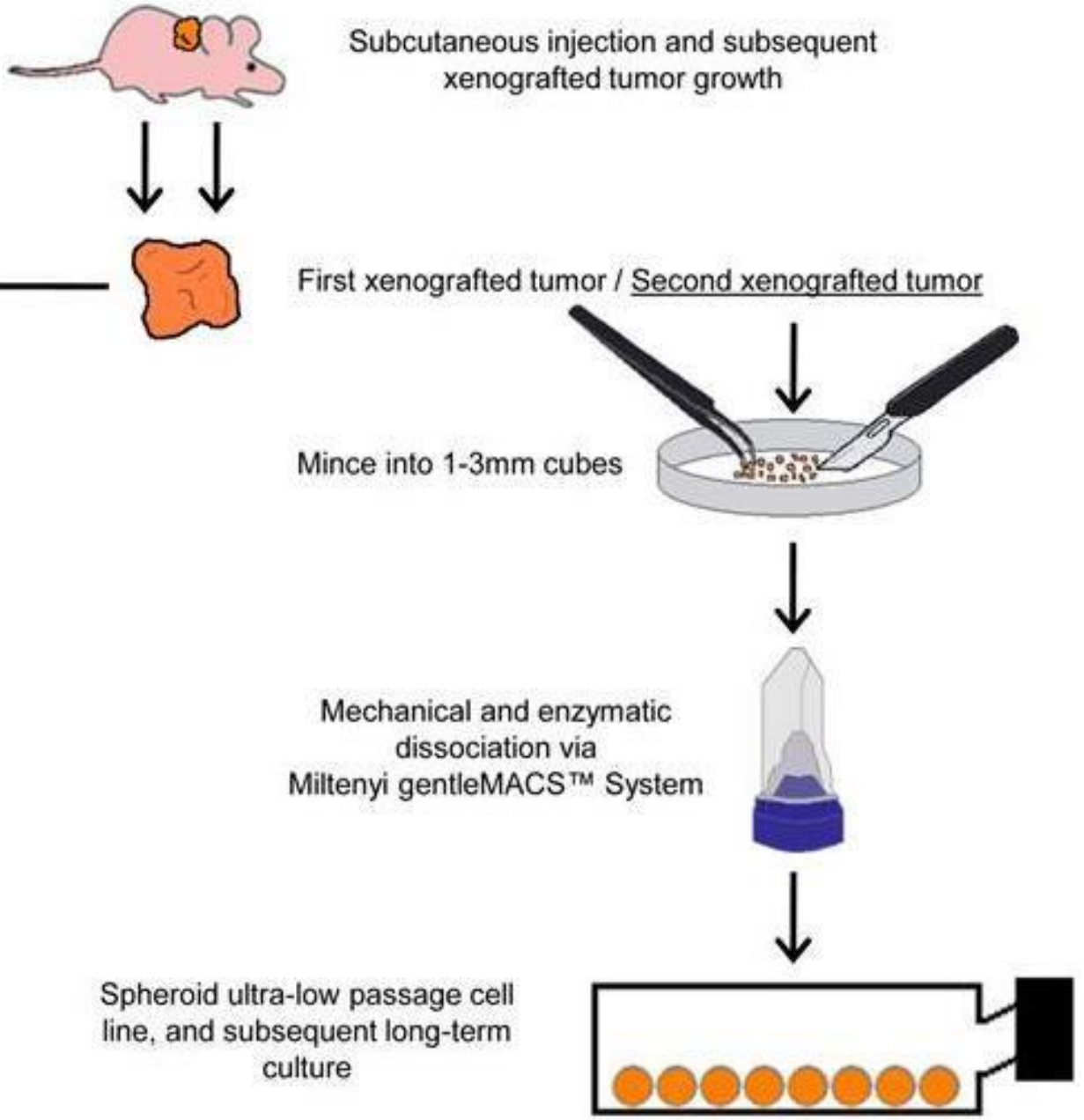


RNA or DNA purify

試劑名稱	廠牌	樣本類型
directzol	ZYMO	組織與細胞均適用
quick RNA	ZYMO	組織與細胞均適用
gentleMACS M Tubes	MACS	耗材

移植瘤解離步驟:

若移植瘤為鼠源細胞株需使用 Mouse dissociation kit，若是移植瘤為人源細胞株則需使用human dissociation kit.



Transfusion. 2015 Dec;55(12):2864-73. doi: 10.1111/trf.13277. Epub 2015 Sep 9.

A new standardized clinical-grade protocol for banking human umbilical cord tissue cells.

Fazzina R[1], Mariotti A[2], Procoli A[2], Fioravanti D[3], Iudicone P[3], Scambia G[2], Pierelli L[3,4], Bonanno G[2].

Author information

1 InScientiaFides Foundation, Republic of San Marino; and.
2 Institute of Gynecology and Obstetrics, Sacred Heart Catholic University.
3 Immunohematology and Transfusion Medicine, San Camillo Forlanini Hospital.
4 Department of Experimental Medicine, Sapienza University, Rome, Italy.

Abstract

BACKGROUND: Mesenchymal stromal cells (MSCs) isolated from human umbilical cord tissue (UCT) can be considered the perfect candidates for cell-based therapies and regenerative medicine. UCT-derived MSCs can be cryogenically stored in cell banks and expanded as needed for therapeutic uses.

STUDY DESIGN AND METHODS: We developed a new method for UCT-MSC isolation, cryopreservation, and expansion, following all criteria required by a stem cell bank. UCT-MSCs were isolated either by manual dissociation (MM) or by a semiautomatic dissociation system (SAM). In both protocols UCTs were treated enzymatically using Type IV collagenase good manufacturing practices (GMP) graded and hyaluronidase (medicinal product). Isolated UCT-MSCs were cryopreserved and analyzed after thawing for phenotype; for proliferation rate; and for their osteogenic, adipogenic, and chondrogenic differentiation capabilities.

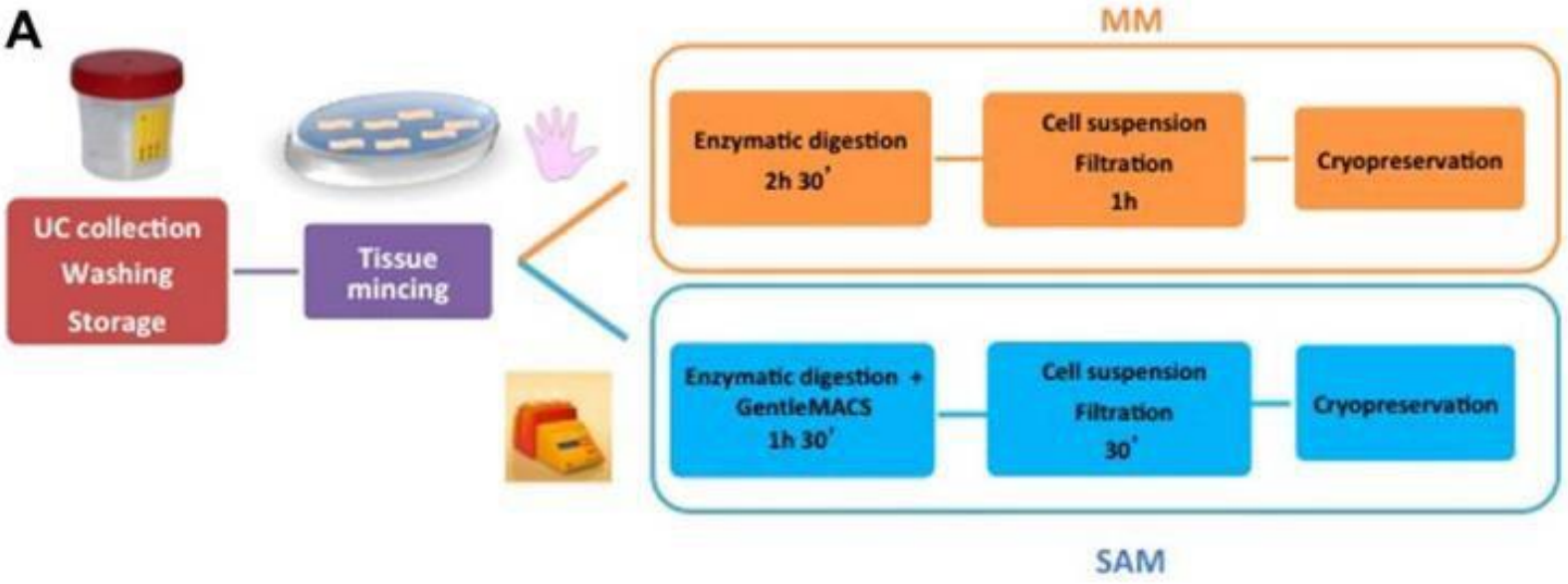
RESULTS: We found that SAM reduced the time of tissue enzyme exposure and enabled us to obtain a homogeneous single-cell suspension deprived of tissue fragments. The isolated cells in both groups showed high expression of MSC markers CD105, CD73, and CD90 and similar differentiation capabilities, phenotype, and proliferation potential. Moreover, the final yield of MSCs was comparable between the two techniques.

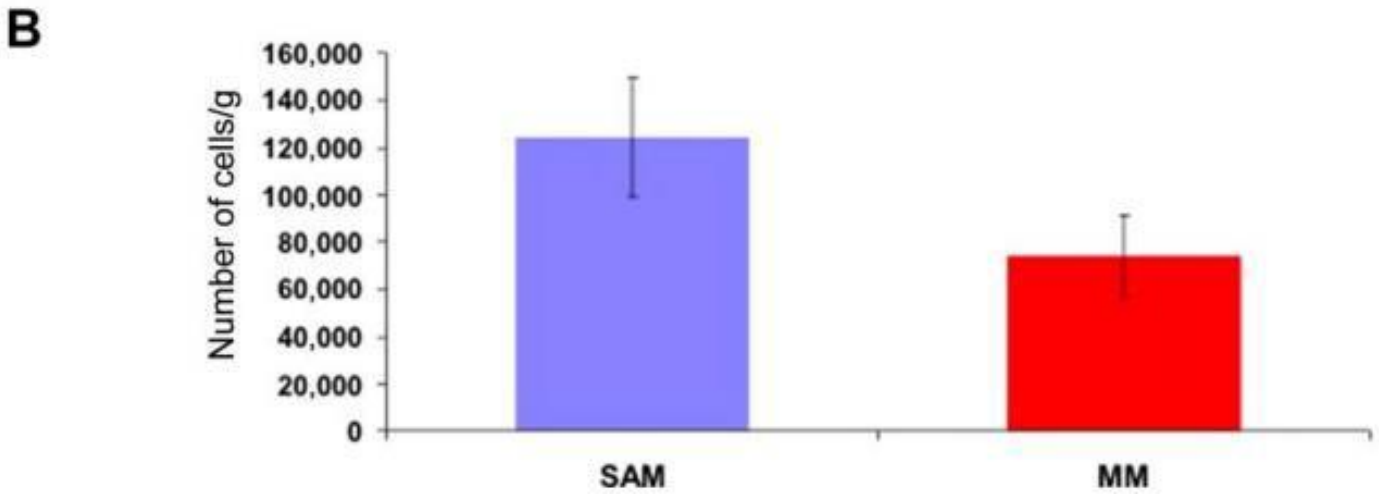
CONCLUSION: In this study, we have established a reliable and standardized protocol to isolate UCT-MSCs from UCT for cell banking purposes. Processing the whole umbilical tissue with GMP-graded enzymes using a semiautomatic dissociator allowed us to obtain a single-cell suspension product with a known number of isolated cells that can be cryopreserved right after isolation and thawed as needed for expansion and clinical use.

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Transfusion. 2015 Dec;55(12):2864-73.

UCT processing and UCTC collection





半自動　　手動

Transfusion. 2015 Dec;55(12):2864-73.

Cell prepartion from tumor tissue – workflow

Miltenyi Biotec

Tissue dissociation



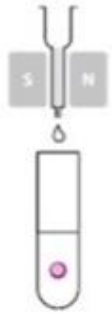
Filtration



RBC lysis



Dead cell removal

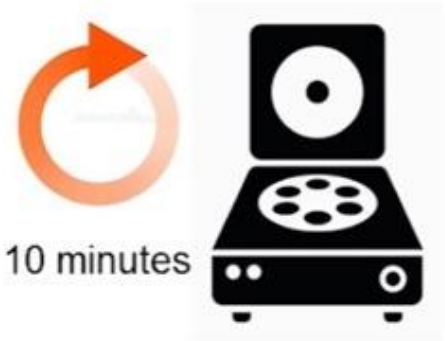


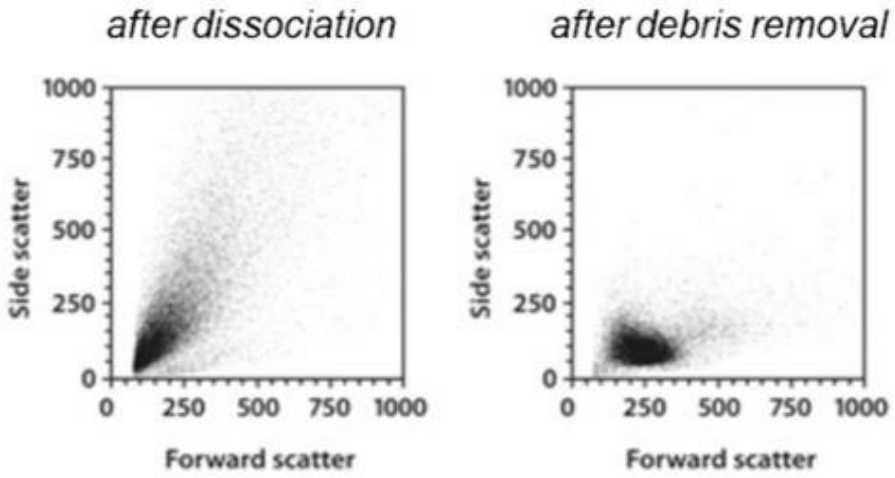
Sample clearing



Debris Removal Solution

- Ready-to-use density gradient reagent
- Fast and effective removal of cell debris
- Applying full acceleration and full brake during centrifugation

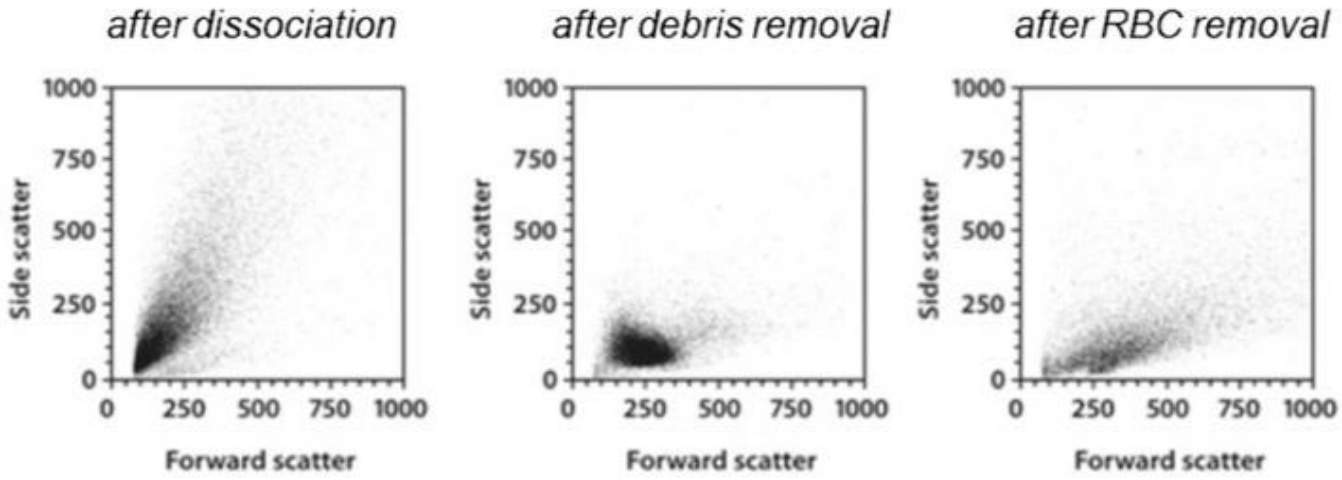




Adult rat heart

Red Blood Cell Removal Solution

- Optimal lysis of erythrocytes
- Minimal effects on cells in human, mouse, or rat whole blood, in bone marrow and in single-cell suspensions from tissue preparations



Adult rat heart

Dead Cell Removal Kit

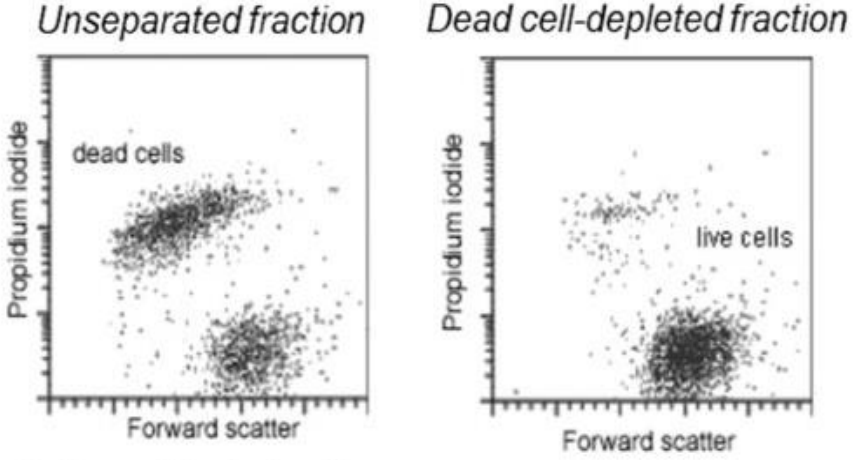
- Ready-to-use MicroBeads
- Fast and straightforward solution to eliminate dead cells from cell culture or tissue preparation
- Pure viable cells within 25 minutes



autoMACS Pro Separator



Manual system



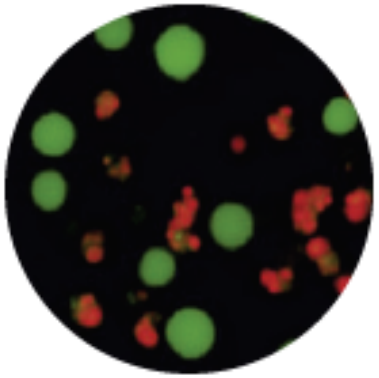
Cultured Jurkat cells

Dead Cell Removal kit

10x technical note 推薦使用 MACS dead cell removal kit

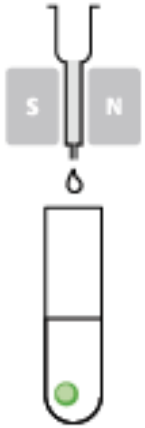
- 去除死細胞後提高樣品潔淨度
- 去除死細胞後提高靶細胞計數的準確性
- 去除死細胞後Library 複雜性增加
- 去除死細胞後線粒體基因數量減

1



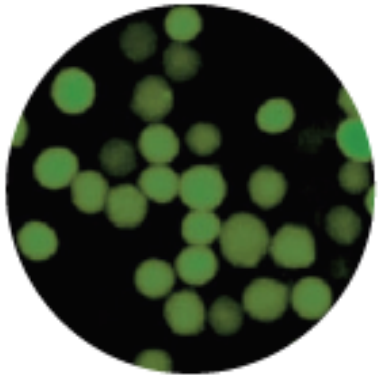
ready-to-use 磁珠直接與樣品反應

2



經過分選柱後，死細胞會被滯留在管柱內，流出的溶液為活細胞

3



收集後的活細胞可直接做培養或後續分析

10x GENOMICS®

TECHNICAL NOTE

Removal of Dead Cells from Single Cell Suspensions Improves Performance for 10x Genomics® Single Cell Applications

MACS Miltenyi Biotec

INTRODUCTION

10x Genomics® Single Cell Protocols require suspensions of viable, single cells as input (*Single Cell Protocols – Cell Preparation Guide* - CG00053). The removal of dead cells and other contaminants from single cell suspensions is critical to obtaining high quality data. Depending on the sample type and sample preparation method, the fraction of dead cells in a single cell suspension can vary significantly. Dead cells easily lyse, resulting in the release of ambient RNA. This cell-free RNA can contribute to the background noise of the assay and will compromise the quality of single cell data. This Technical Note discusses the impact of non-viable cells in single cell suspensions on single cell data genomics quality. In this Technical Note we compare datasets generated from single cell suspensions containing varying amounts of non-viable cells to highlight the importance of removing dead cells from single cell suspensions prior to use with the Single Cell 3' v2 Protocol.

METHOD

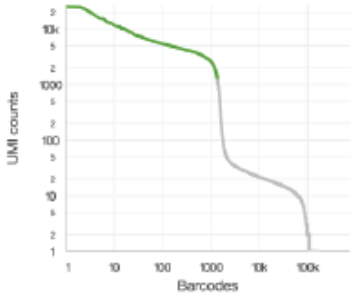
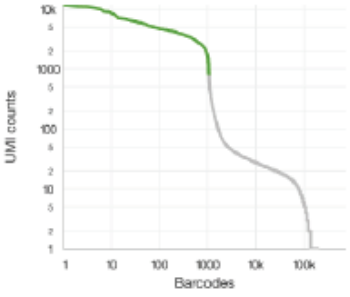
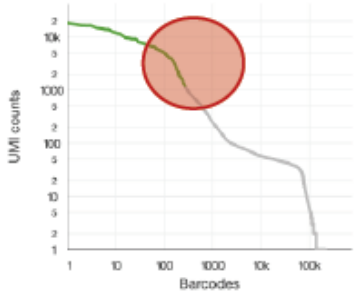
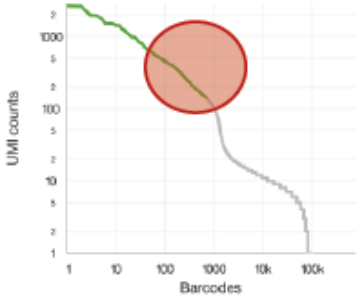
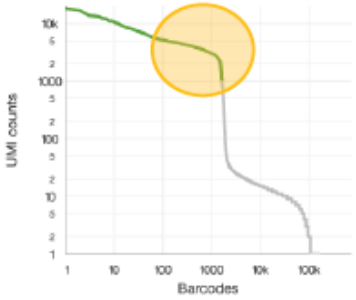
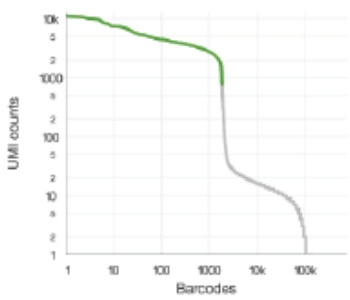
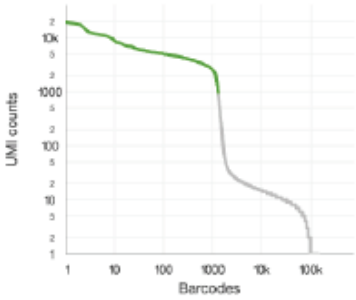
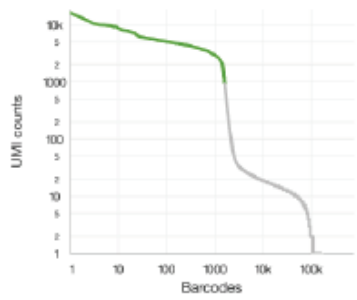
We prepared single cell suspensions from peripheral blood mononuclear cells (PBMCs) from a healthy donor by following the Demonstrated Protocol *Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing* – CG00039. The single cell suspension was then split into four groups for further processing. In order to elevate the fraction of dead cells we treated each cell suspension as follows:

PBMC suspension #1 (Control):	Cells loaded immediately for partitioning after sample preparation
PBMC suspension #2 (24 h at RT):	Cells left at room temperature (RT) in PBS for 24 h
PBMC suspension #3 (Digitonin Low):	Cells treated with 5 ng/ml Digitonin. Cell suspension resulted in < 5% viable cells and was mixed with >90% viable PBMCs at a 1:1 ratio
PBMC suspension #4 (Digitonin High):	Cells treated with 5 ng/ml Digitonin. Cell suspension resulted in < 5% viable cells and was mixed with >90% viable PBMCs at a 5:1 ratio

實驗結果

	Control	24 h at RT	Digitonin Low	Digitonin High
Pre Dead Cell Removal	85%	50%	50%	20%
Post Dead Cell Removal	85%	86%	85%	82%

Cell viability for each PBMC suspension assessed with Trypan Blue and the Countess II Automated Cell Counter after sample treatment and removal of dead cells with the Dead Cell Removal Protocol.

Treatment	Control	24 h at RT	Digitonin Low	Digitonin High
Pre-Dead Cell Removal				
Post-Dead Cell Removal				

Barcode Rank Plot for 8 samples pre and post Dead Cell Removal treatment. Plotted is the distribution of barcode counts (x-axis) and the corresponding total UMI (Unique Molecular Identifier) counts (y-axis). Cell-containing partitions (barcodes on x-axis) are shown in green. Background partitions are shown in grey. Red circle indicates lack of steep drop-off. Yellow circle indicates one example of clear steep drop-off.

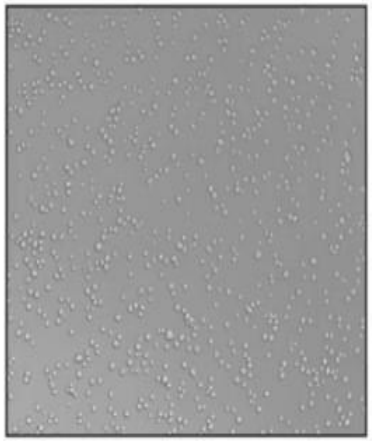
The Chromium™ System from 10X Genomics is based on droplet-based technology



Sample Quality Is Critical

10X GENOMICS

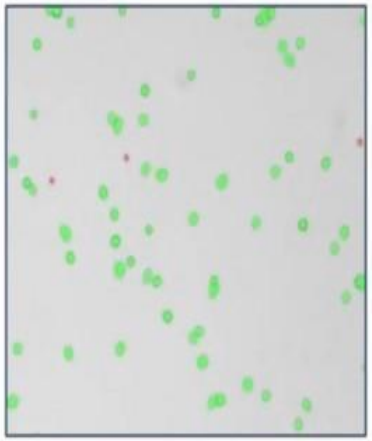
Clean Suspension



Challenges

- Dissociation
- Aggregates/clumps
- Subcellular debris
- Free-floating mRNA

High Viability



Challenges

- Dying cells have reduced RNA content
- Free-floating mRNA

Gentle Handling



Challenges

- Pipetting technique
- Buffer selection
- Time

Tumor Dissociation Workflow

10x GENOMICS

Dissociate → **Filter** → **Lyse RBCs** → **Remove Dead Cells**



Octo Dissociator with Heater

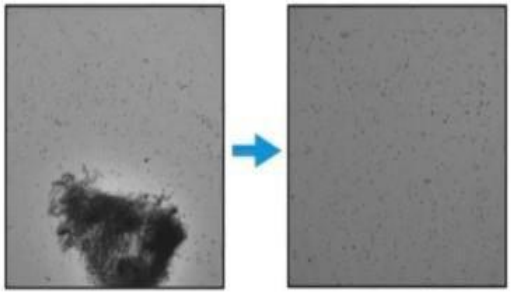


Tumor Dissociation Kit, mouse





MACS SmartStrainer





Red Blood Cell Lysis Solution



Dead Cell Removal Kit



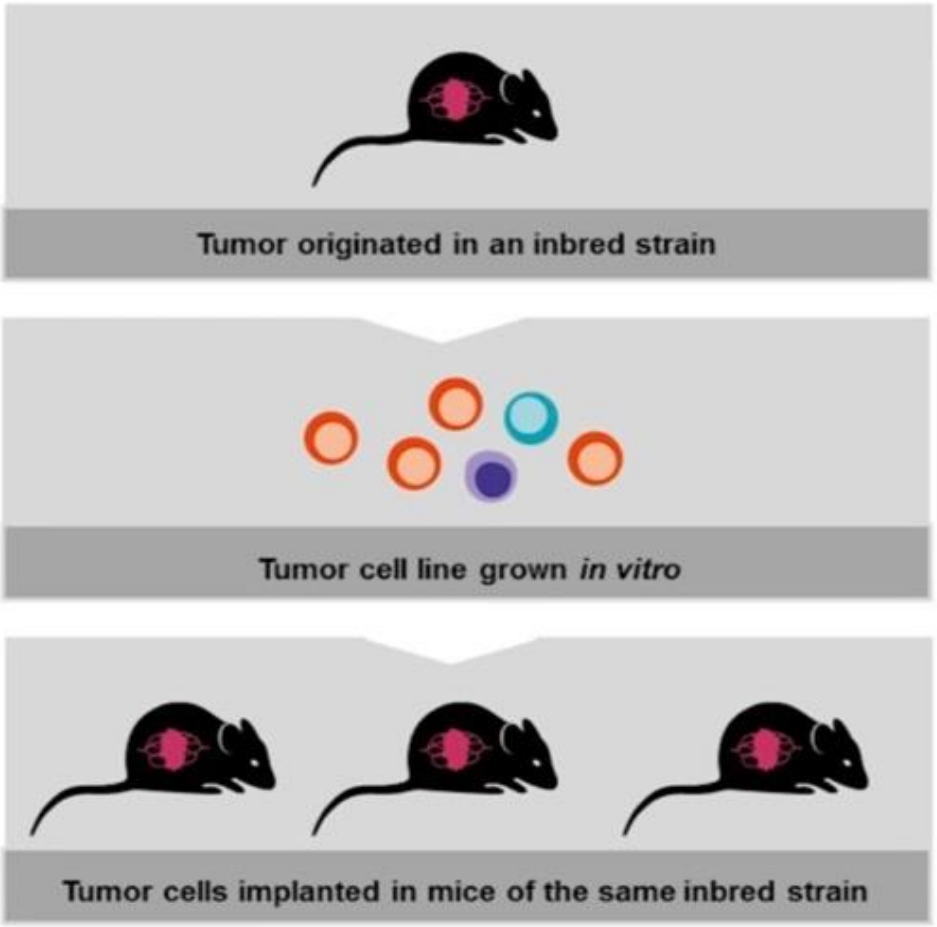
MS/LS Columns

Best Quality Cell Suspension

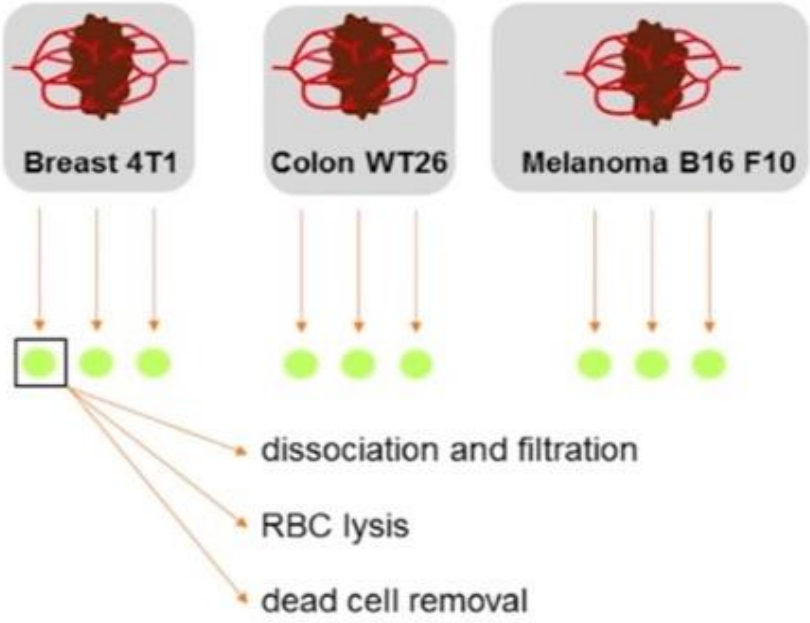


Study design

Syngeneic Mouse Tumor Model



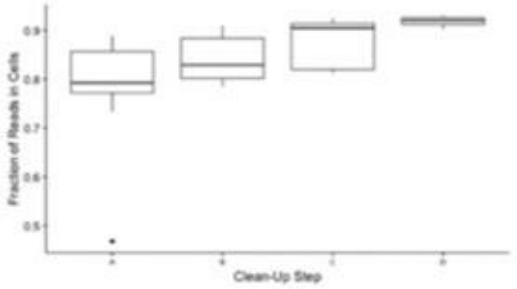
Experimental setup



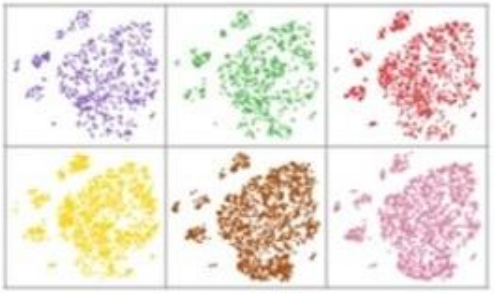
9 Tumors: 3 tissues, 3 reps
47 sequencing libraries
84,042 single cells sequenced

Study goals

- **Performance**



- **Reproducibility**



- **Tumor heterogeneity**

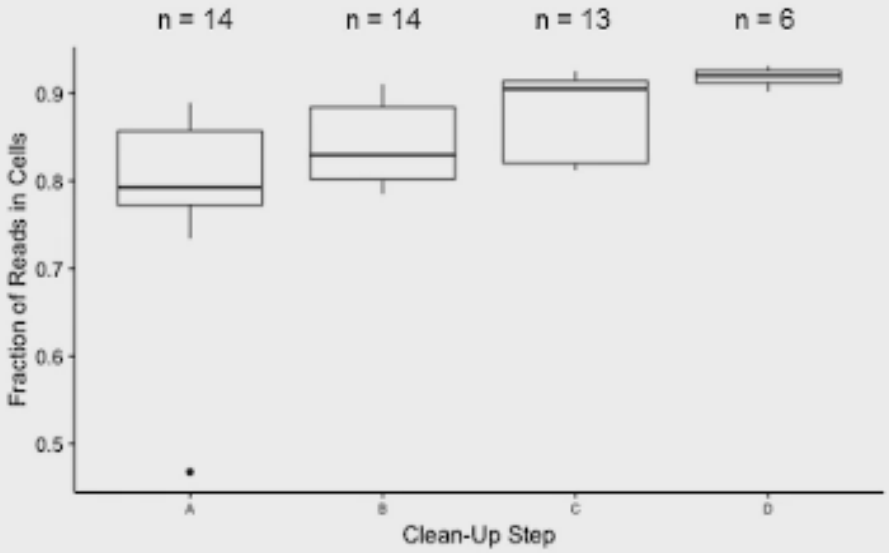


Performance

Increase in Cleanliness and Complexity

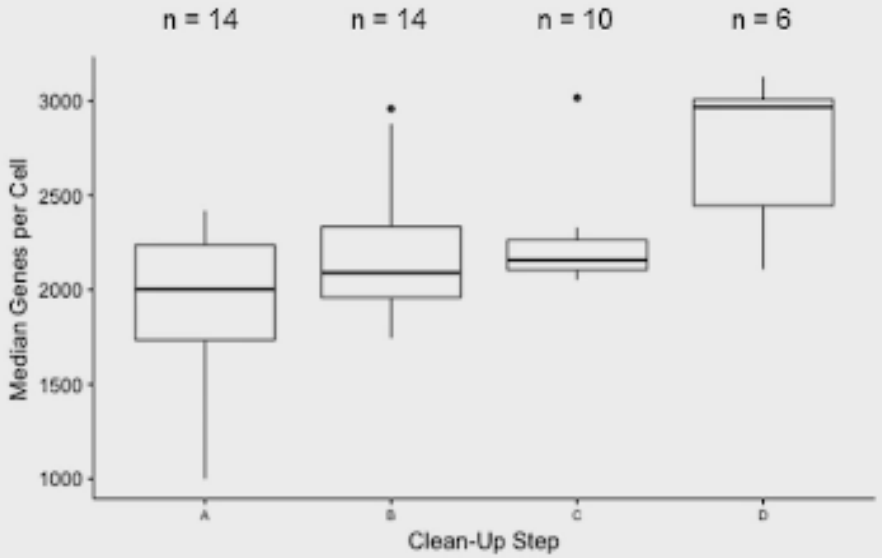
Cell Ranger™ Software Pipeline

Library Cleanliness



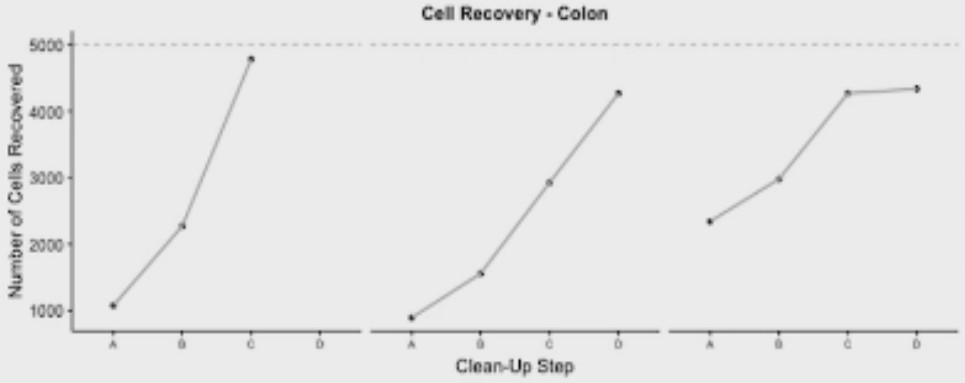
A = Post Filtration | B = Post RBC Lysis | C, (D) = Post Dead Cell Removal(s)

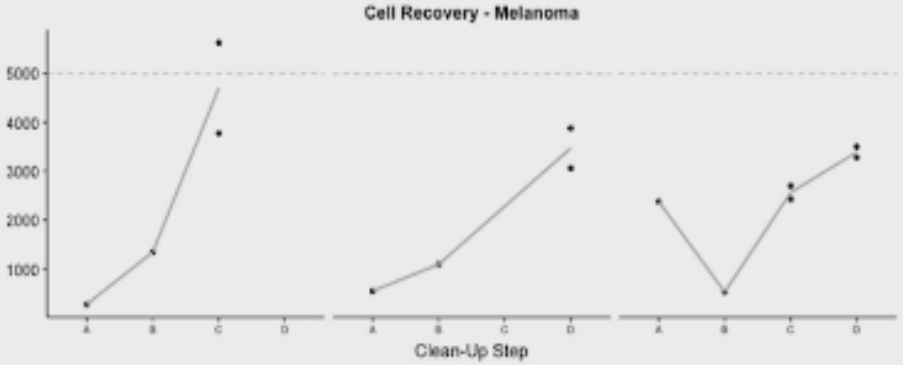
Library Complexity

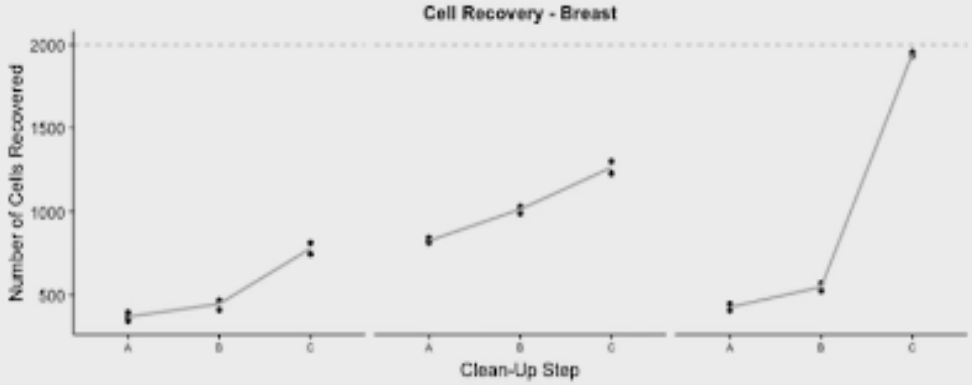


20,000 reads per cell (low seq. depth)

Substantial Increase in Cell Recovery







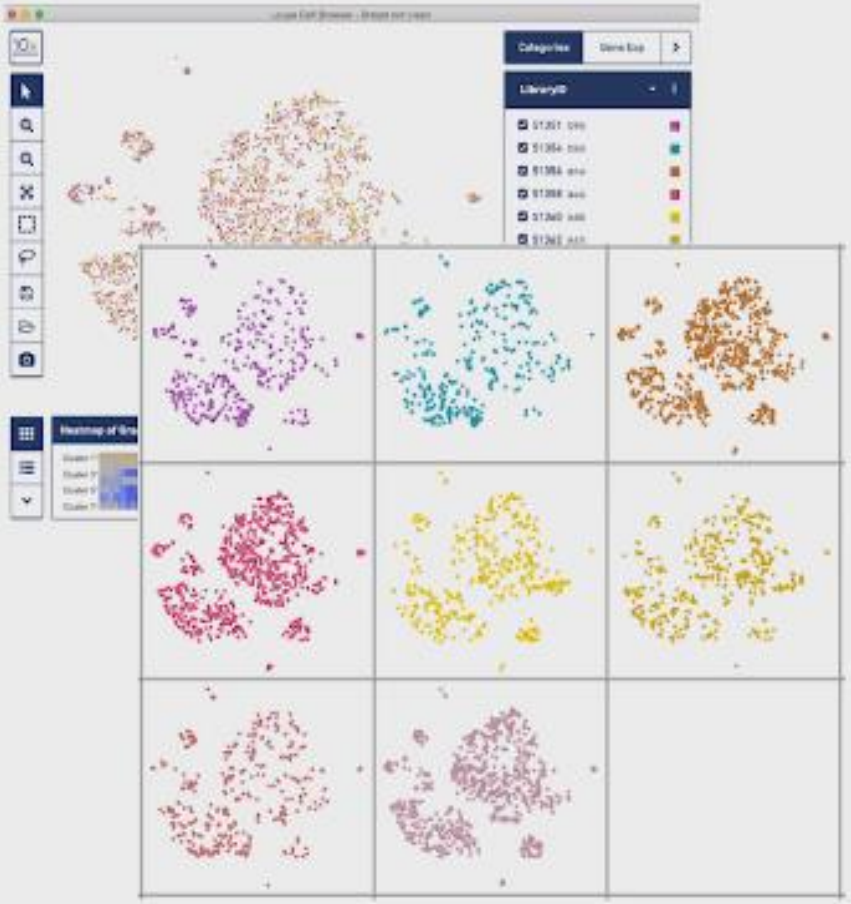
A = Post Filtration | B = Post RBC Lysis | C, (D) = Post Dead Cell Removal(s)

Breast tumors – reproducible results

10x GENOMICS

MACS Miltenyi Biotec

Filtered Only



Plus RBC and Dead Cell Removal



Loupe Cell Browser™ Software

Colon tumors – reproducible results

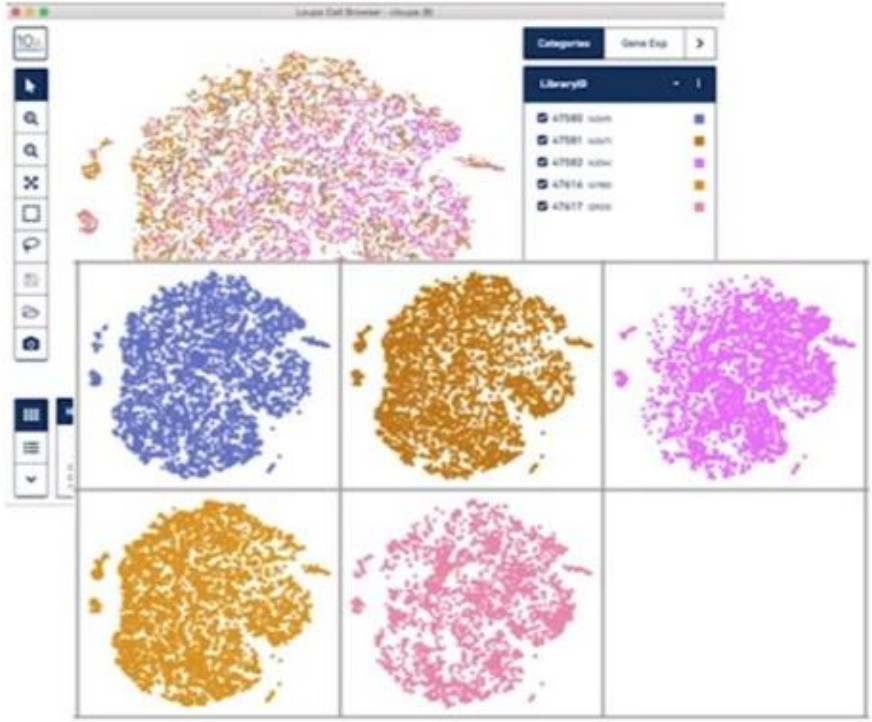
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Filtered Only



Plus RBC and Dead Cell Removal



Melanoma – additional clean-ups increase the reproducibiliy of results

10x GENOMICS

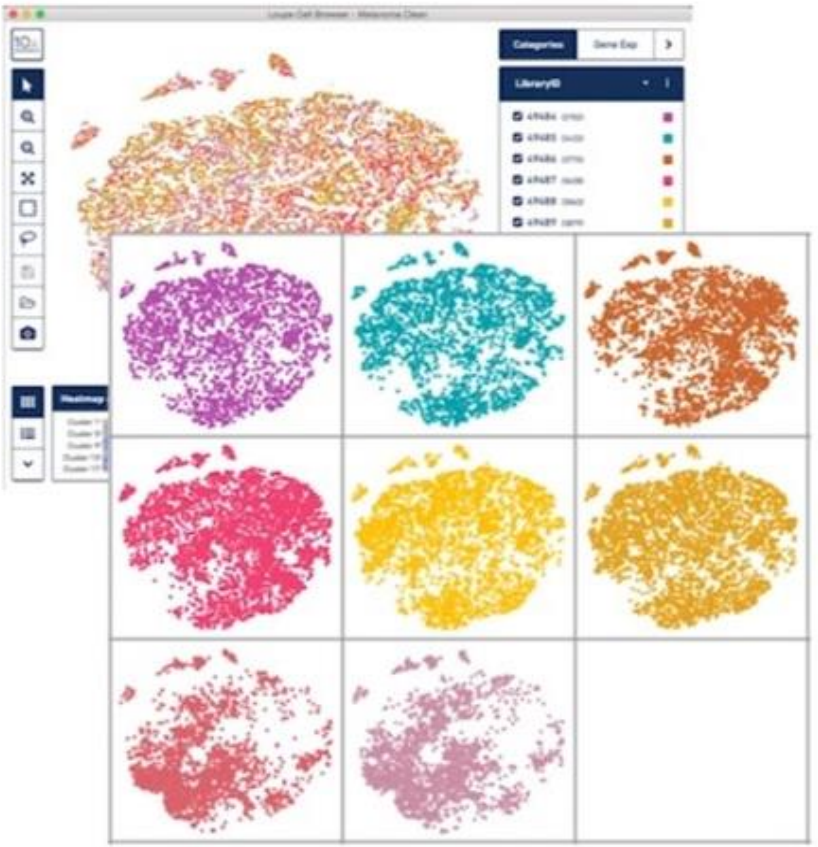
MACS Miltenyi Biotec

Filtered Only

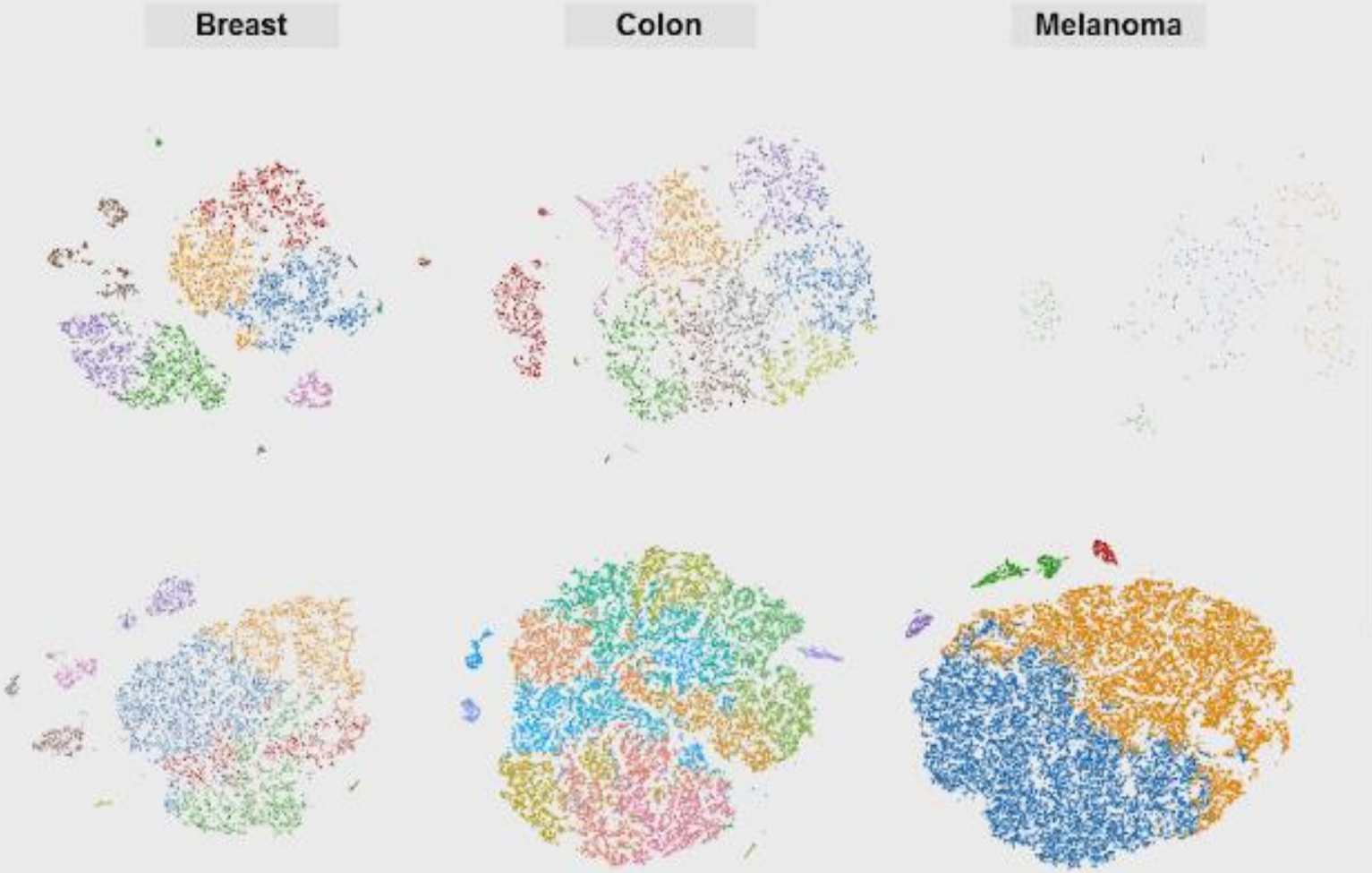


poor reproducibility

Plus RBC and Dead Cell Removal



Tumor heterogeneity – analysis of cell types



gentleMACS 儀器操作

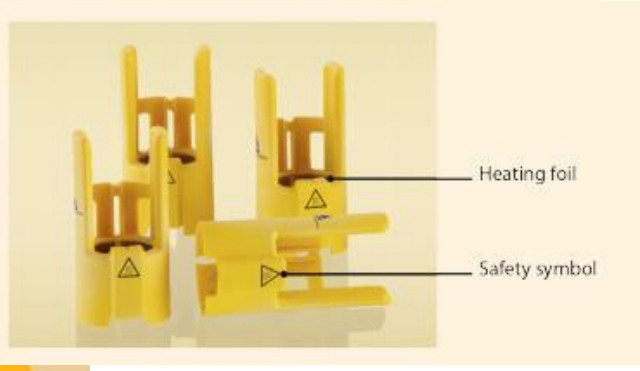
Miltenyi Biotec

操儀器操作介紹:

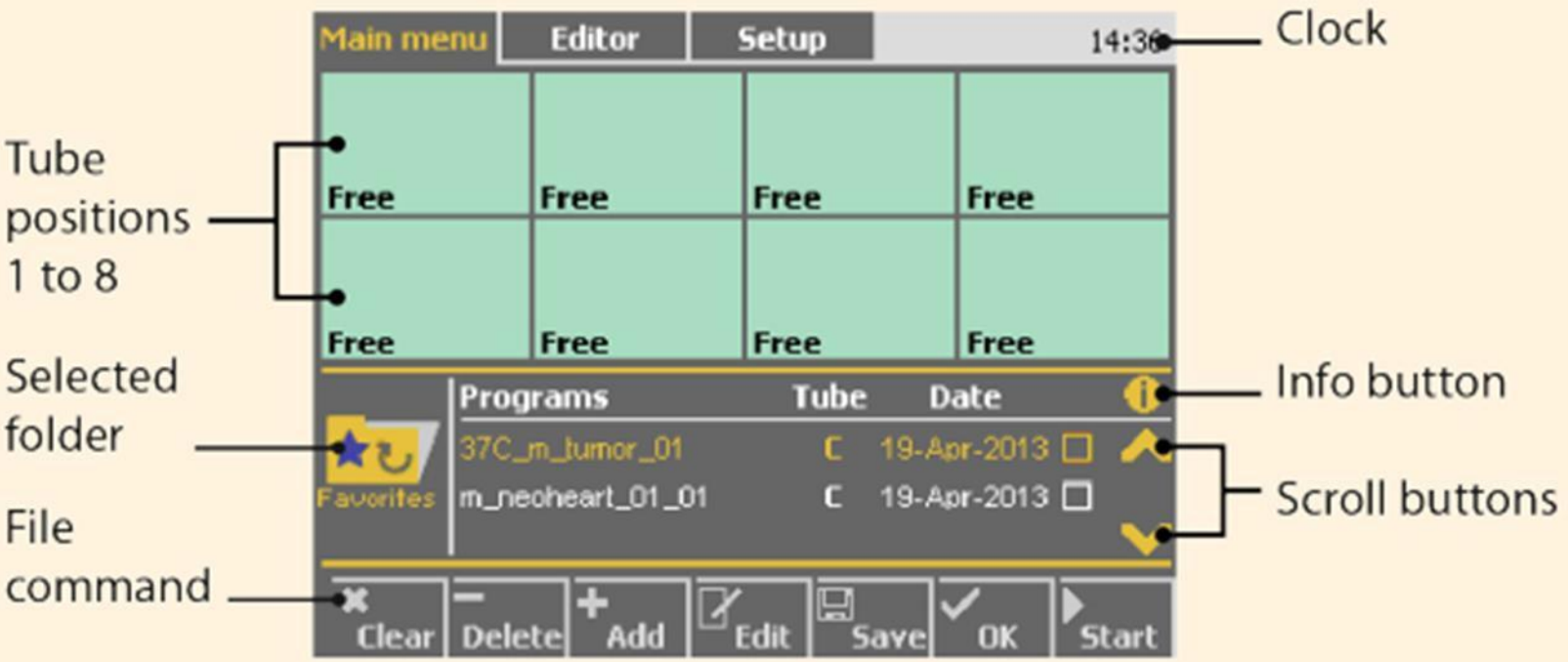
Miltenyi Biotec







儀器操作介紹


Main menu
Editor
Setup
14:36
Clock
Tube positions 1 to 8
Free
Selected folder
Programs
Tube
Date
Info button
Favorites
37C_m_tumor_01
C
19-Apr-2013
m_neoheart_01_01
C
19-Apr-2013
Scroll buttons
File command
Clear
Delete
Add
Edit
Save
OK
Start

儀器操作介紹

Miltenyi Biotec

Icon	Folder	Description
	Miltenyi	The **Miltenyi** folder contains gentleMACS Programs provided by Miltenyi Biotec. The lock on the Miltenyi folder indicates that programs in this folder cannot be edited or deleted.
	Templates	The **Templates** folder contains gentleMACS Programs provided by Miltenyi Biotec. Programs in this folder can be edited but must be renamed and saved in a User folder.
	User - 1	Five **User** folders are available to manage user-defined programs.
	User - 2	
	User - 3	
	User - 4	
	User - 5	
	Favorites	Links of programs in the **Favorites** folder enable quick access.
	USB stick	A USB stick can be installed to transfer programs to and from a folder.

儀器操作介紹


Main menu
Editor
Setup
14:49
1
Free
2
Selected
37C_m_
or_01
3
05m:00s
Ready
m_neol
_01_01
4
01m:00s
Ready
m_neol
_01_01
5
Running
m_neol
_01_01
6
00m:03s
Done
m_neol
_01_01
7
00m:58s
Aborted
8
Stand by
Programs
Tube
Date
Favorites
37C_m_tumor_01
C
19-Apr-2013
m_neoheart_01_01
C
19-Apr-2013
Clear
Delete
Add
Edit
Save
OK
Start

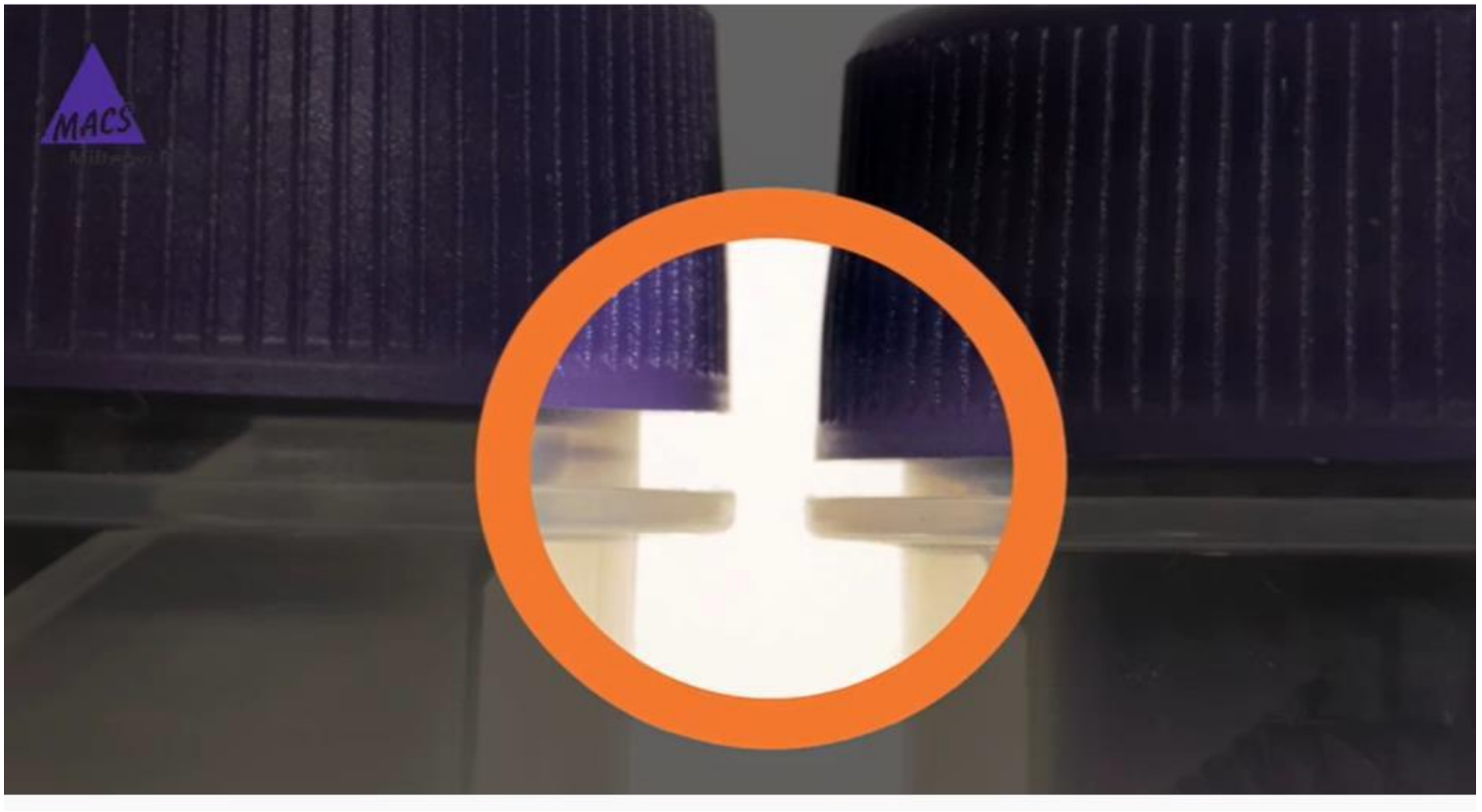
gentleMACS dissociator

操作注意事項與儀器清潔保養



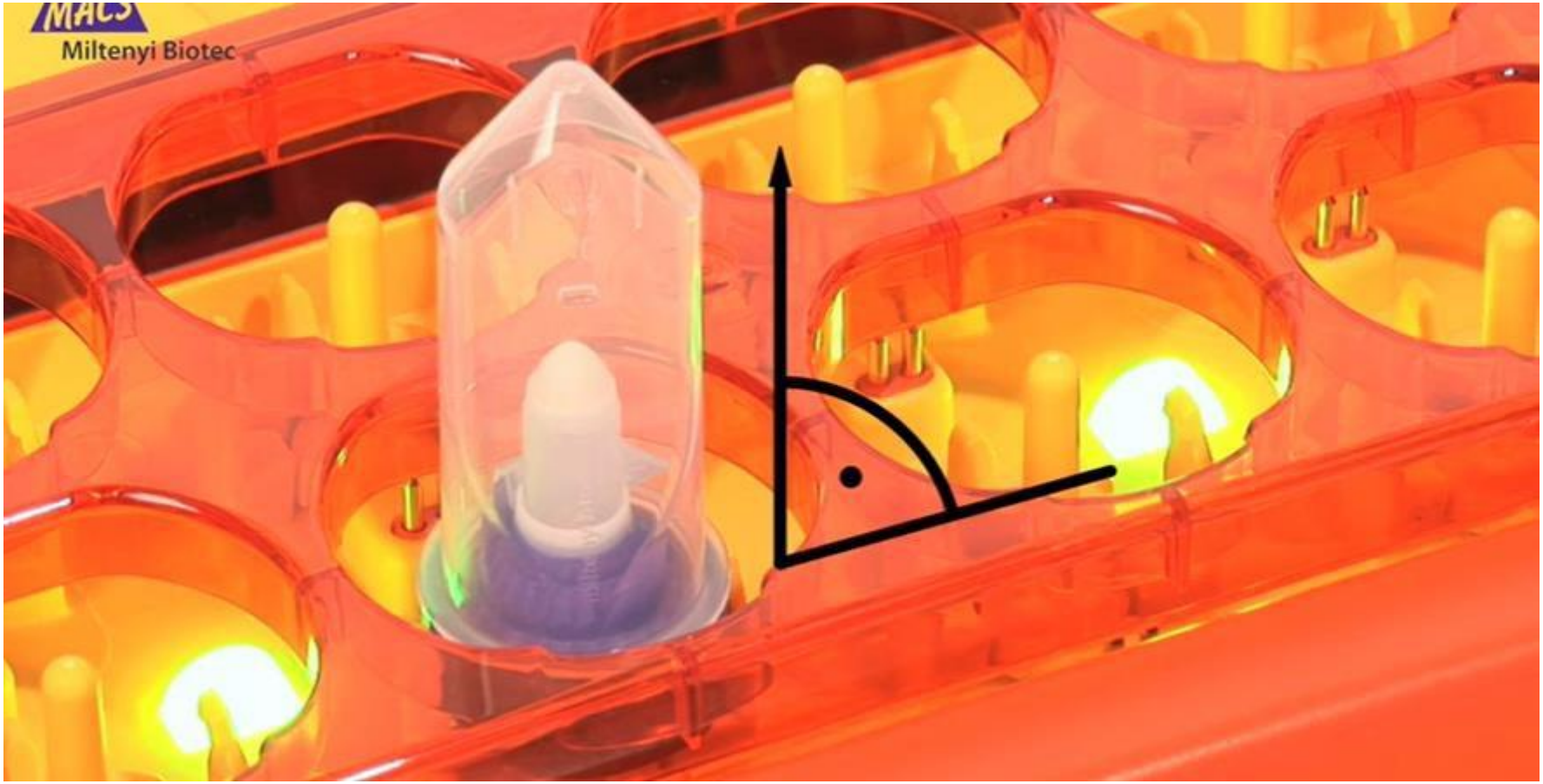
操作注意事項:

1. 不論是C-tube 或是M-tube 在放到機器上時一定要轉緊。否則會發生液體洩漏，導致儀器損傷。



操作注意事項:

2.完成後取下tube時，需垂直向上不要左右搖動tube。左右搖晃容易損壞底板上的tube固定架。



儀器清潔保養:

1.清潔儀器前請拔掉電源線

2.移出防護罩

3.移出套筒進行清潔或消毒

4.移出槽蓋進行清潔

5.儀器表面可用含有去污劑的水溶液或者70%乙醇擦拭

6.清潔完成後，按順序組裝各元件

