autoMACS™ Pro Separator

自動細胞純化分選儀

NEW

Now, high quality cell separations are even easier!

J & H 博克科技有限公司

林敬雯

MACS

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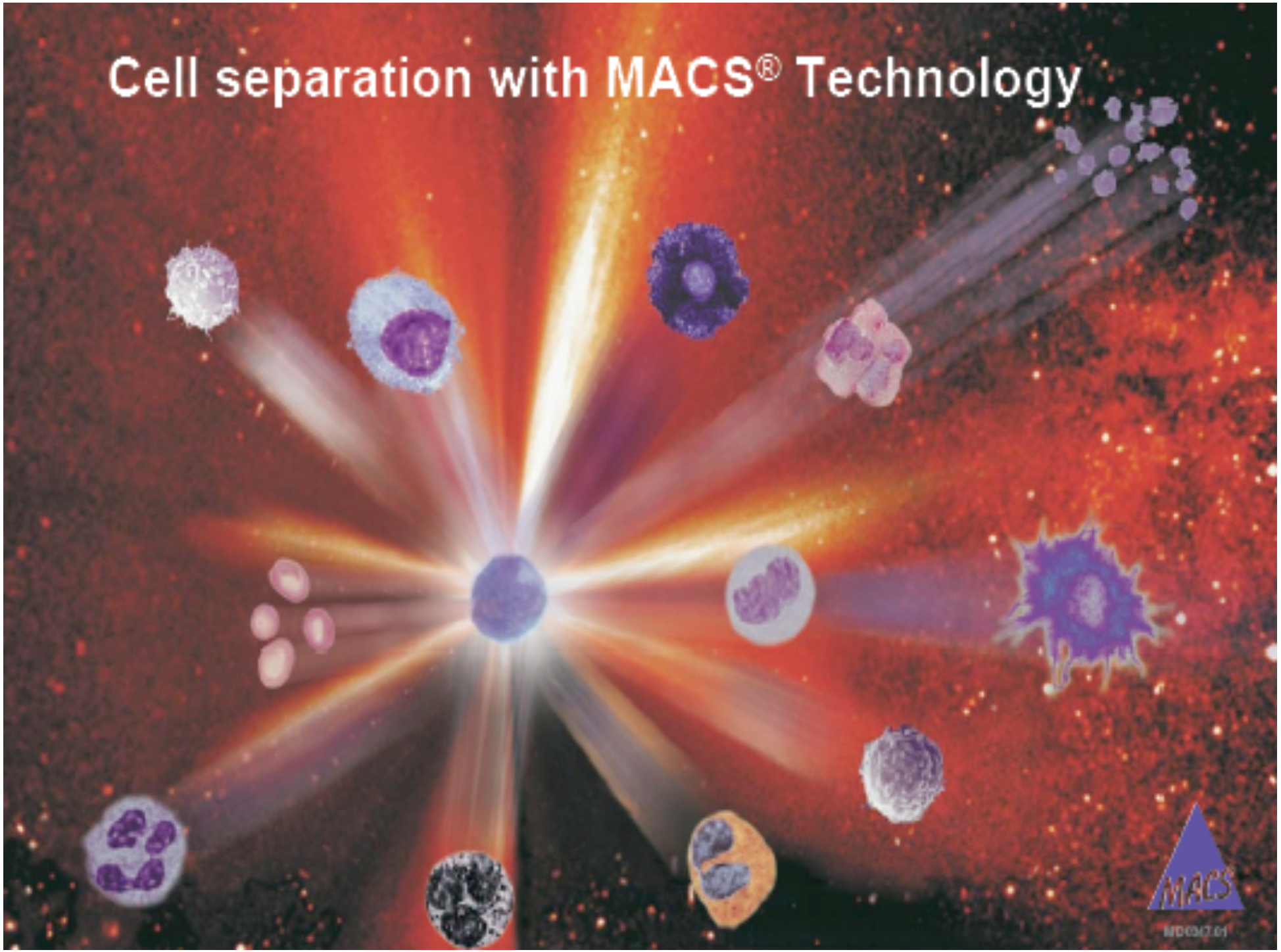
Cell separation with MACS® Technology
MACS

Separation techniques

- physical methods
  - » lysis (e.g. erythrocytes)
  - » filtration (e.g. single cells)
  - » adherence (e.g. nylon wool)
  - » density gradient centrifugation (Ficoll, Percoll)

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Separation techniques

Immunological methods: Flow cytometry

- **Flow sorting time:**
  10 000 cells / sec

= 6 x 10^5 cells / min

= 3.6 x 10^7 cells / h

10^7 cells => 16 min
10^8 cells => 2 h 40 min
10^9 cells => 27 h

- **MACS® sorting time:**
  (autoMACS™ separator)

10^7 cells => 5 min
10^8 cells => 5 min
10^9 cells => 5 min

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MACS Technology

Equipment and reagents

- MACS MicroBeads
- MACS Columns
- MACS Magnet
- Detailed protocol

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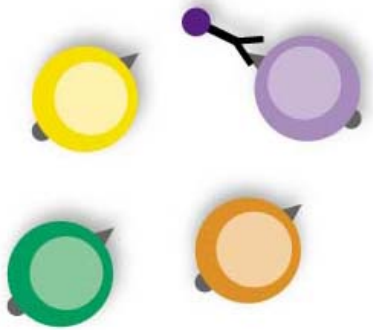
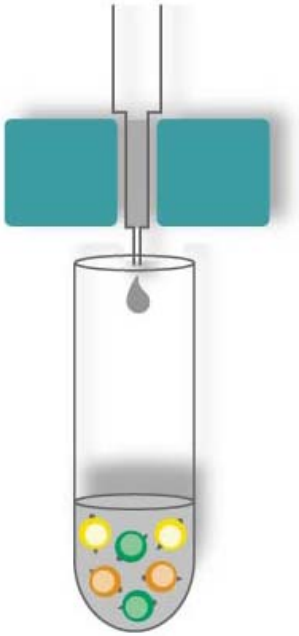
Based on renowned MACS® Technology

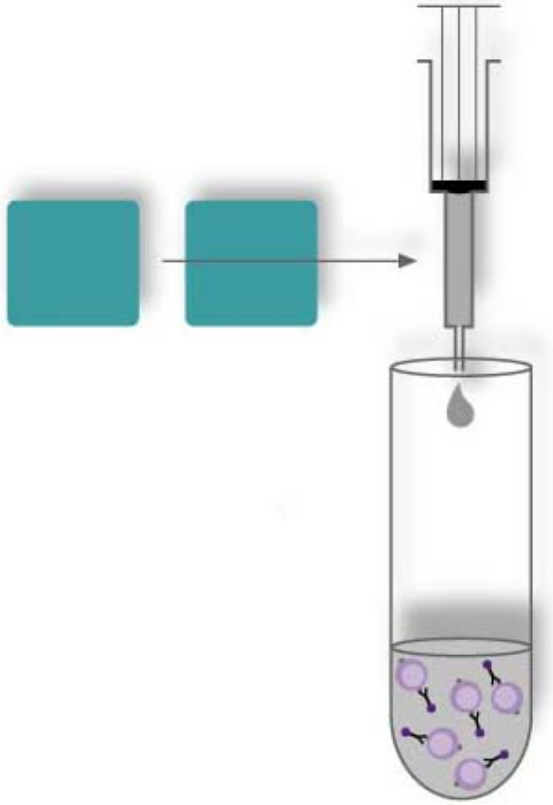
MACS® Magnetic Cell Sorting: reagents, columns, magnets



Magnetic labeling with MACS® MicroBeads



Cells labeled with MACS® MicroBeads are retained in the MACS® Column. Unlabeled cells pass through and are collected as the untouched fraction



Elution of the labeled cell fraction

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Major advantages

- **Small beads: 50 nm**
  - Cell function preserved
  - Flow cytometry compatible

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- Beads 200 times smaller in size than a cell
  (50 nm = virus-sized)
  - Colloidal suspension
  - Short incubation times
    15 min, 4°C => avoid cell activation

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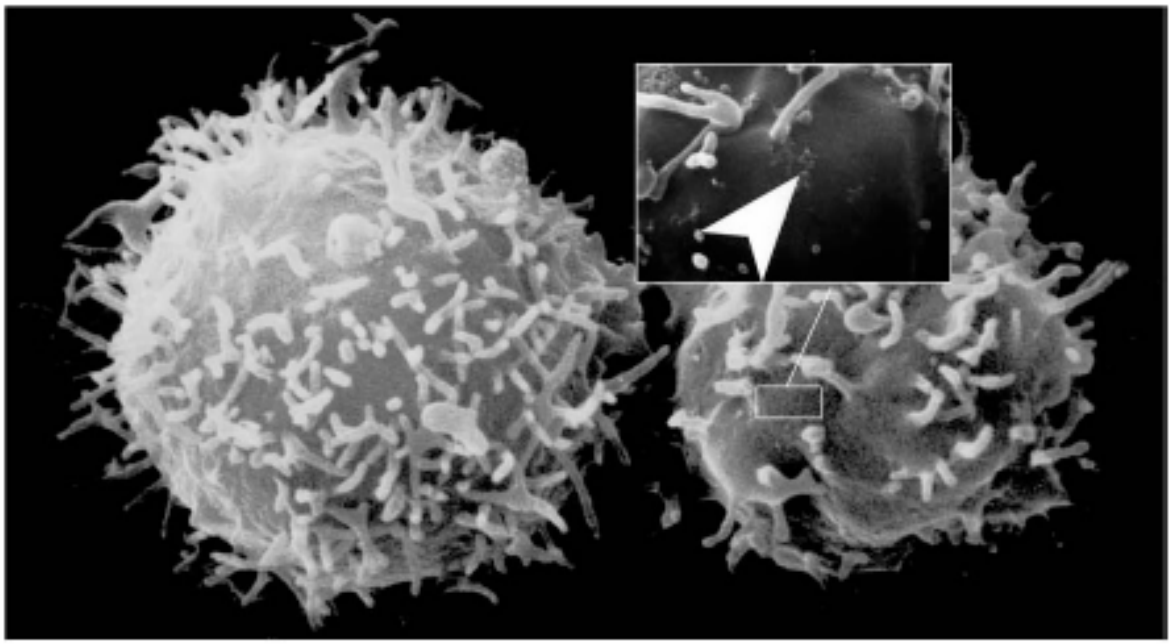
CD8^{+} T cells isolated by MACS® Technology



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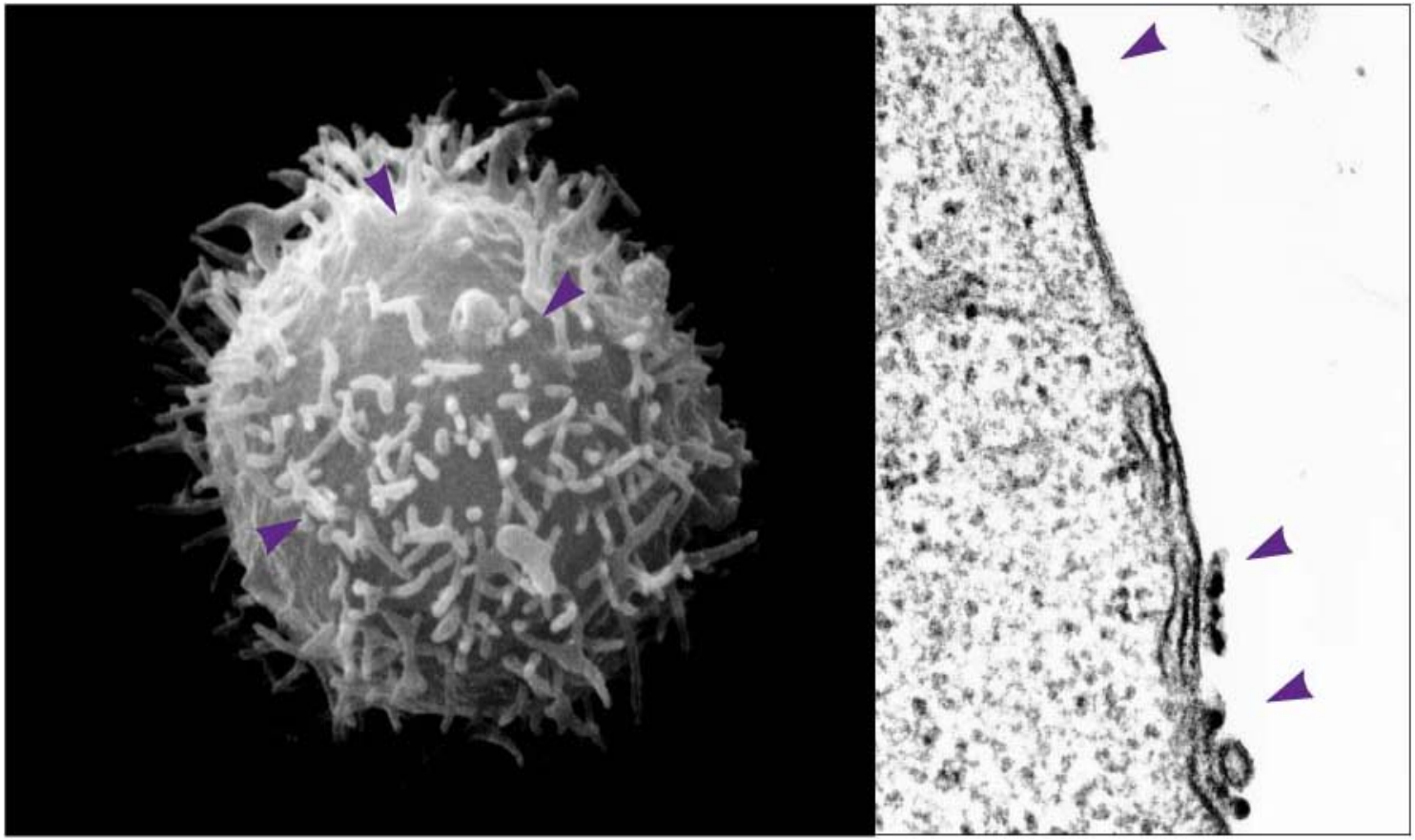
CD8+ T cells isolated by MACS® Technology



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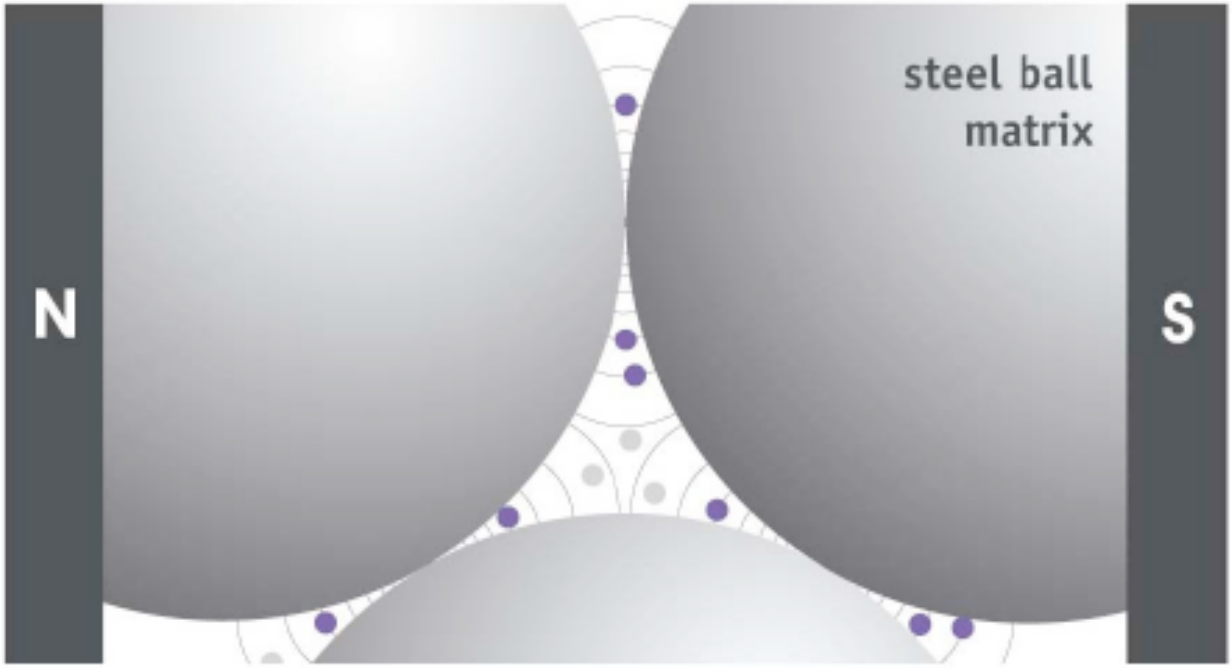
MD0057.03

Magnetic field generated in an MS Column



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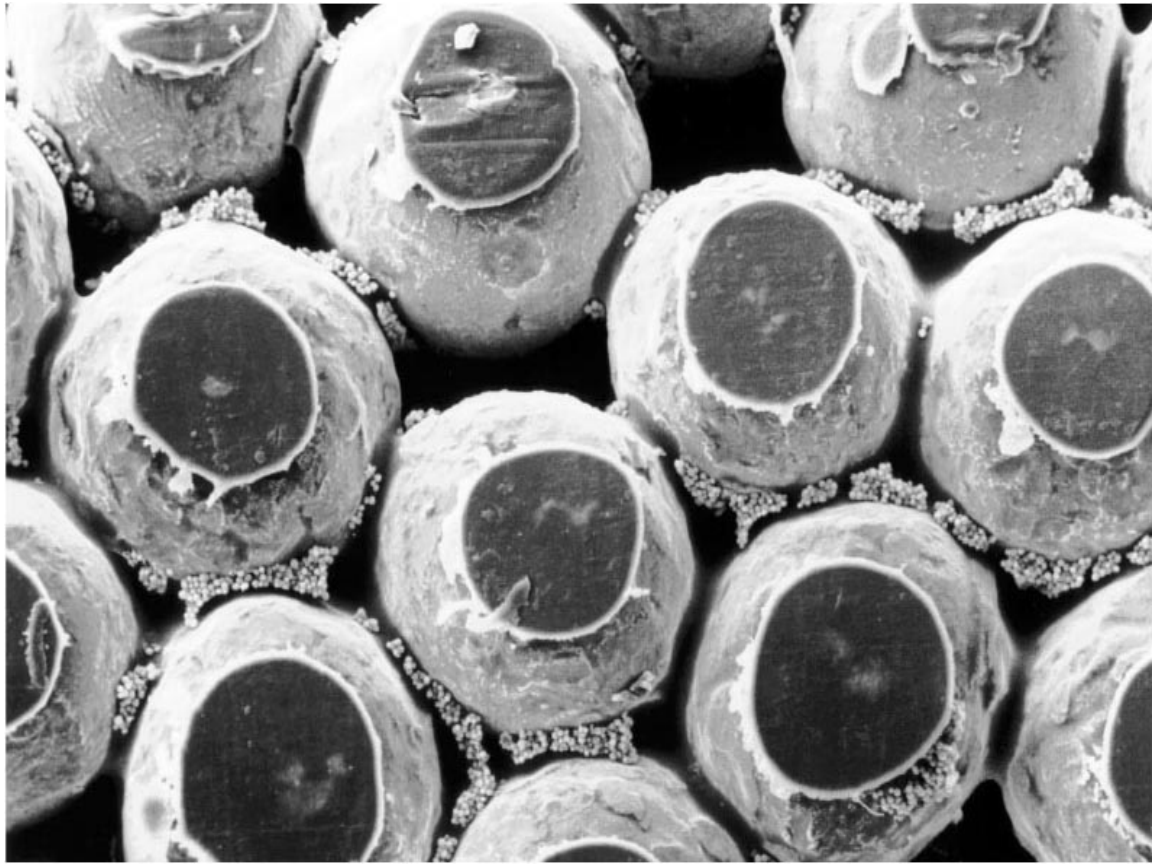
Matrix of an MS Column



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MD0123.03

Major advantages

- Small beads: 50 nm
  - **Cell function preserved**
  - Flow cytometry compatible

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- Straight to experiment or cell culture
  - Non - toxic
  - Biodegradable

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Major advantages

- Small beads: 50 nm
  - Cell function preserved
  - **Flow cytometry compatible**

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- No bead detachment required
  - Only 20-30% of binding sites occupied

Parallel staining with fluorochrome-conjugated antibodies.

  - Decreased flow sorting time

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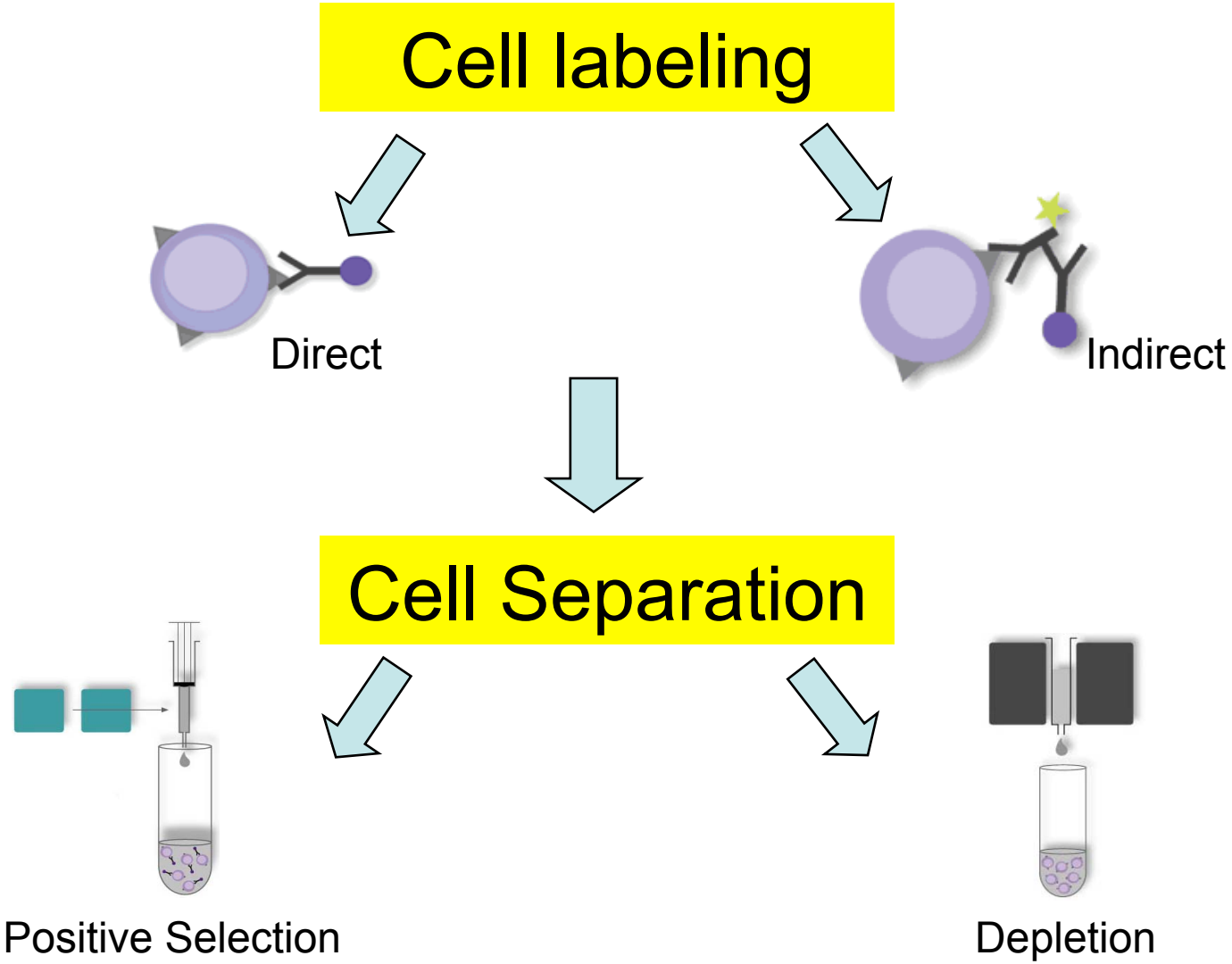
Strategy:



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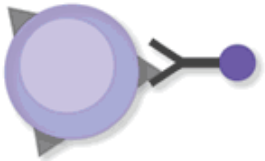
Direct labeling



Cell Separation: Human

Cell Separation: Tumor Cells

Cell Separation:
Non-Human Primate

Cell Separation: Mouse

Cell Separation: Rat

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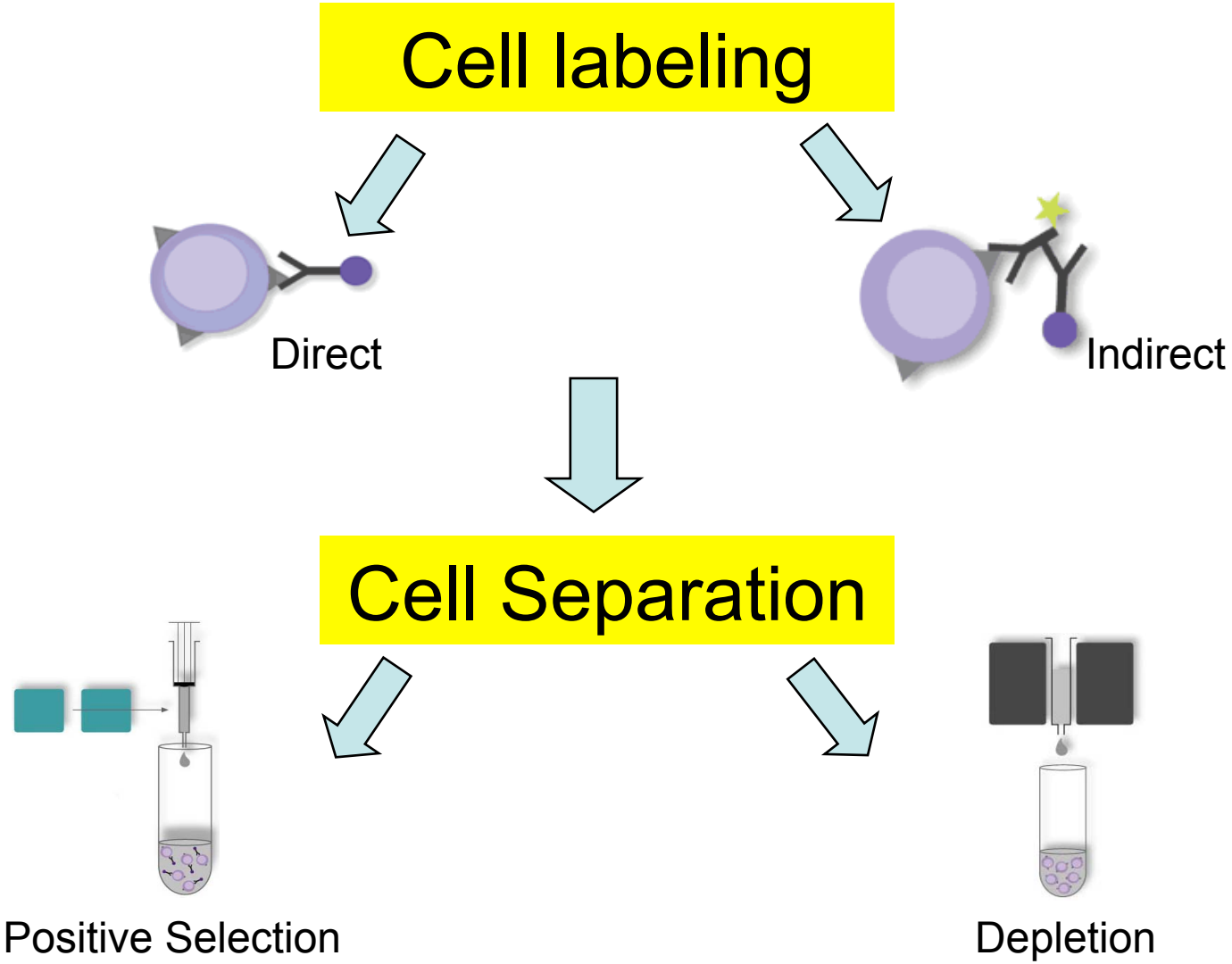
Strategy:



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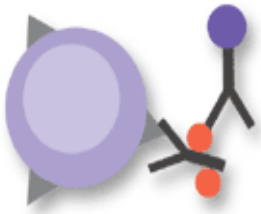
MACS® Indirect MicroBeads-no limits

- When no direct MicroBeads are available
- When an own antibody or ligand is used
- When dimly expressed markers are targeted



Anti-Biotin MicroBeads

Streptavidin MicroBeads

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Indirect magnetic labeling

Primary antibodies:

- unconjugated

  monoclonal (mouse, rat)

  or polyclonal (rabbit)

- or conjugated

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MACS Indirect MicroBeads

Anti-Immunoglobulin MicroBeads

in combination with unconjugated primary antibody

Anti-Fluorochrome MicroBeads

in combination with fluorochrome conjugated primary antibodies

Anti-Biotin MicroBeads or Streptavidin MicroBeads

in combination with biotinylated primary antibodies

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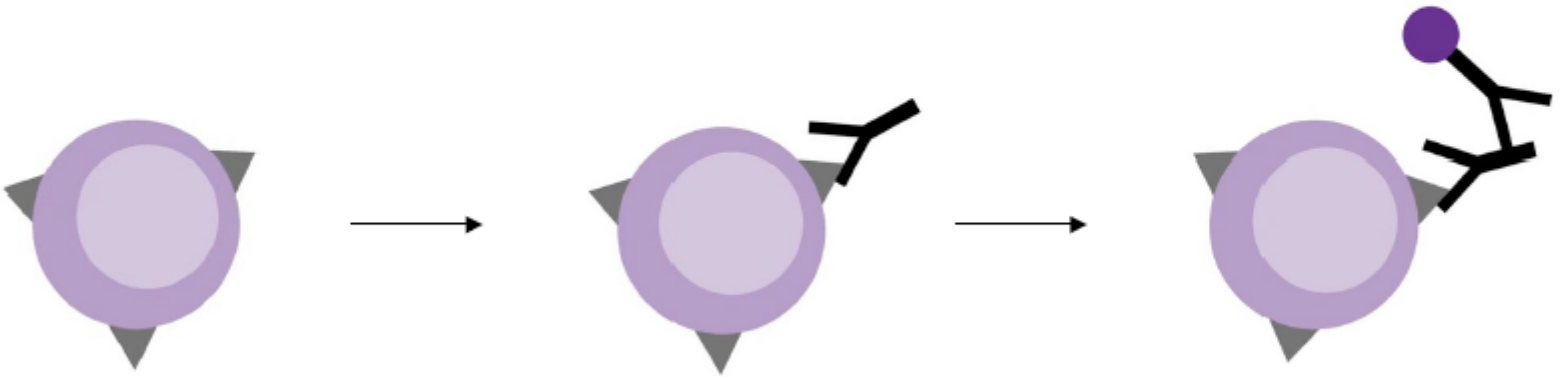
Indirect magnetic labeling



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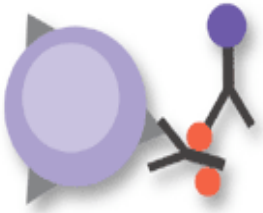
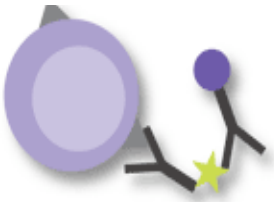
MACS® Indirect MicroBeads-no limits

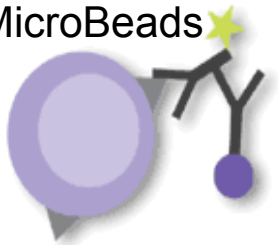
Anti
-Immunoglobulin
MicroBeads



Anti-Biotin MicroBeads



Anti-fluorochrome
MicroBeads



Anti
-Immunoglobulin
MicroBeads

Streptavidin MicroBeads

Antibody MicroBead

Streptavidin MicroBead

Biotinylated antibody

FITC-conjugated antibody

Phycoerythrin (PE)-conjugated antibody

Allophycocyanin (APC)-conjugated
antibody

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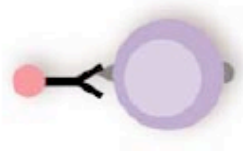
Multiparameter magnetic cell sorting

using MACS® MultiSort MicroBeads

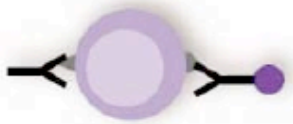
Labeling with MultiSort MicroBeads



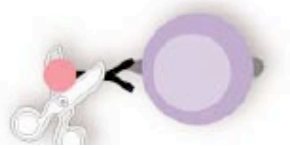
Inhibition of the release reaction

MACS separation

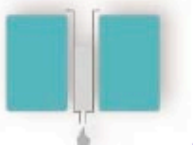
Labeling with MicroBeads according to a second marker



Enzymatic release of the magnetic particle



MACS separation



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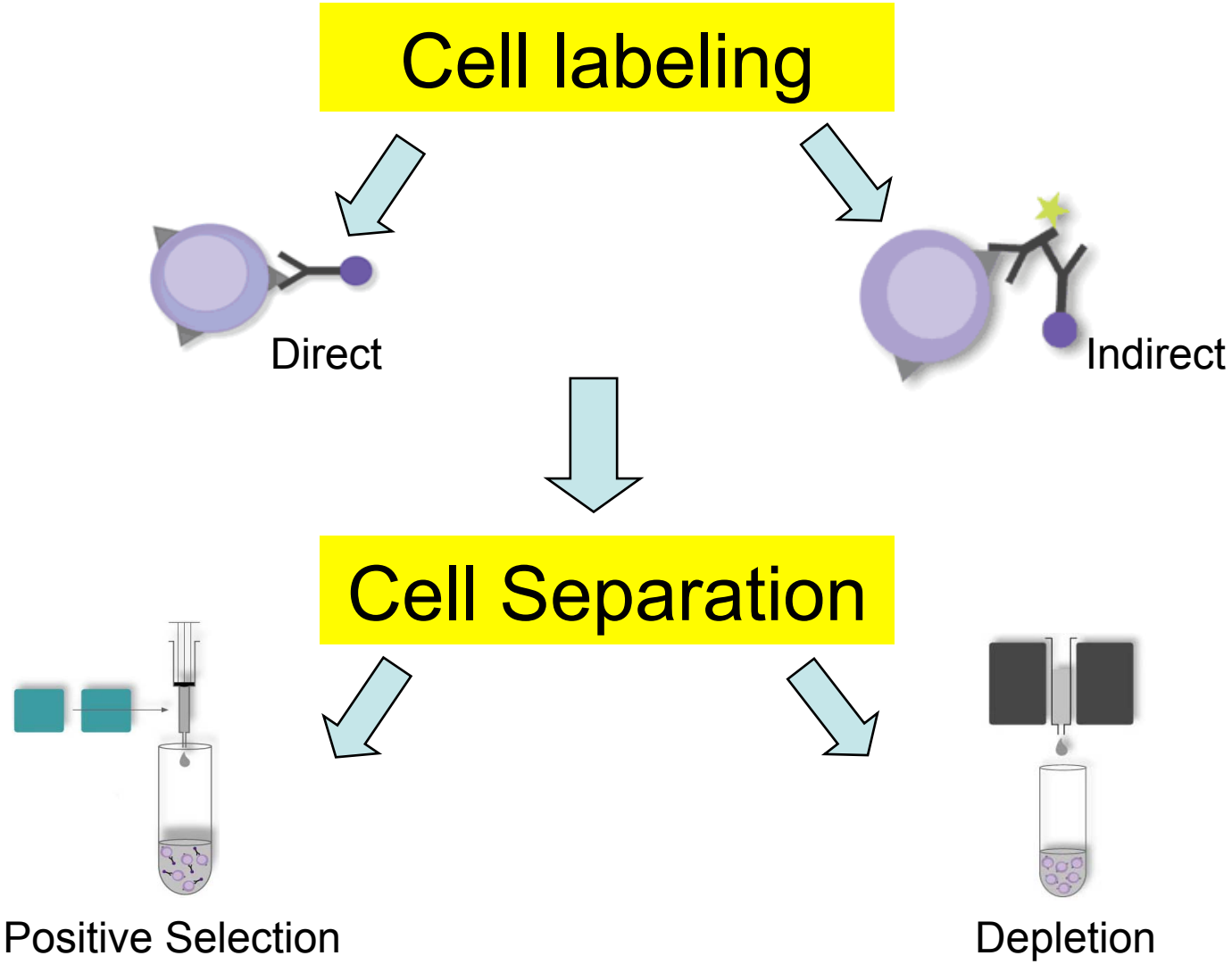
Strategy:



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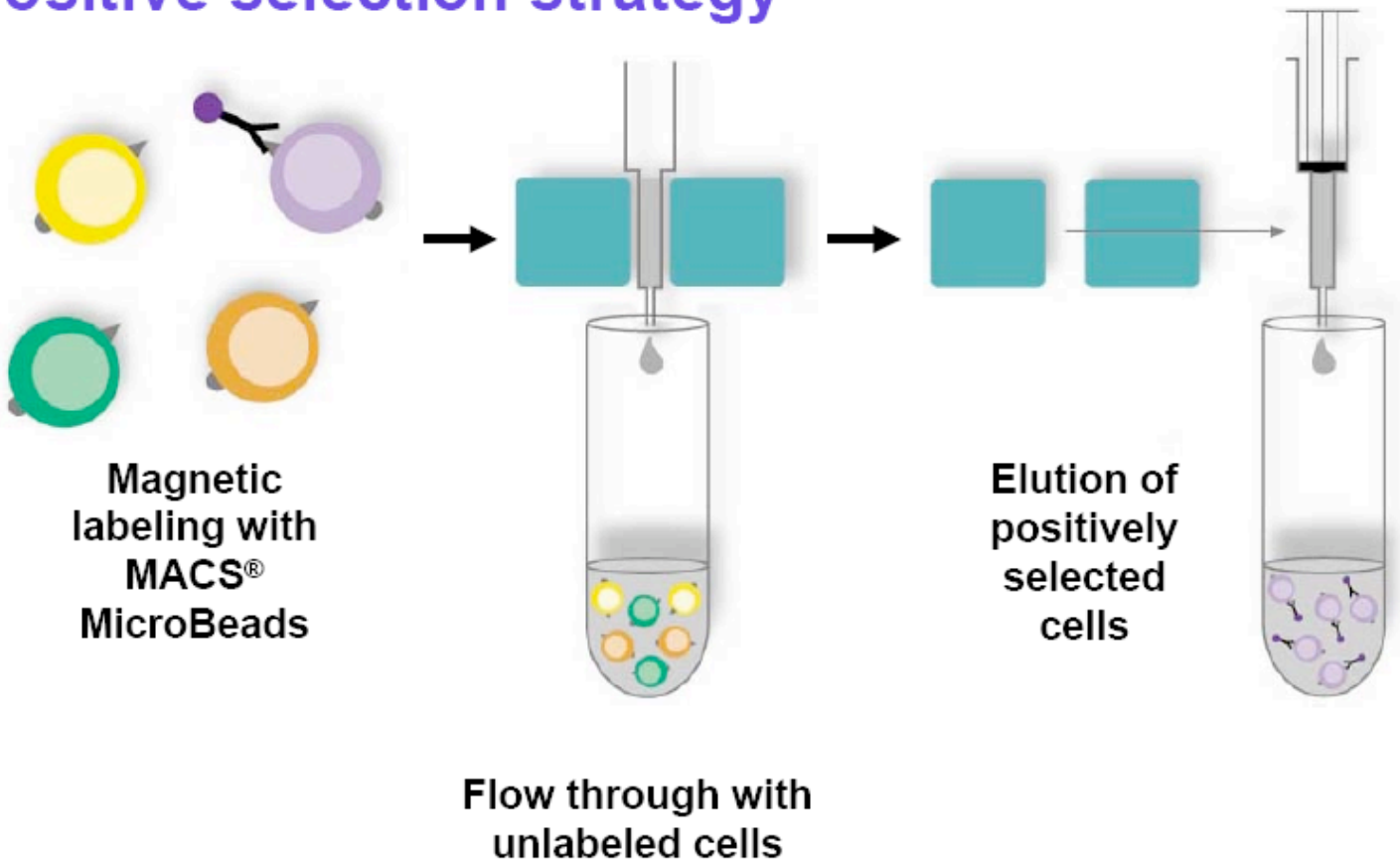
Positive selection strategy



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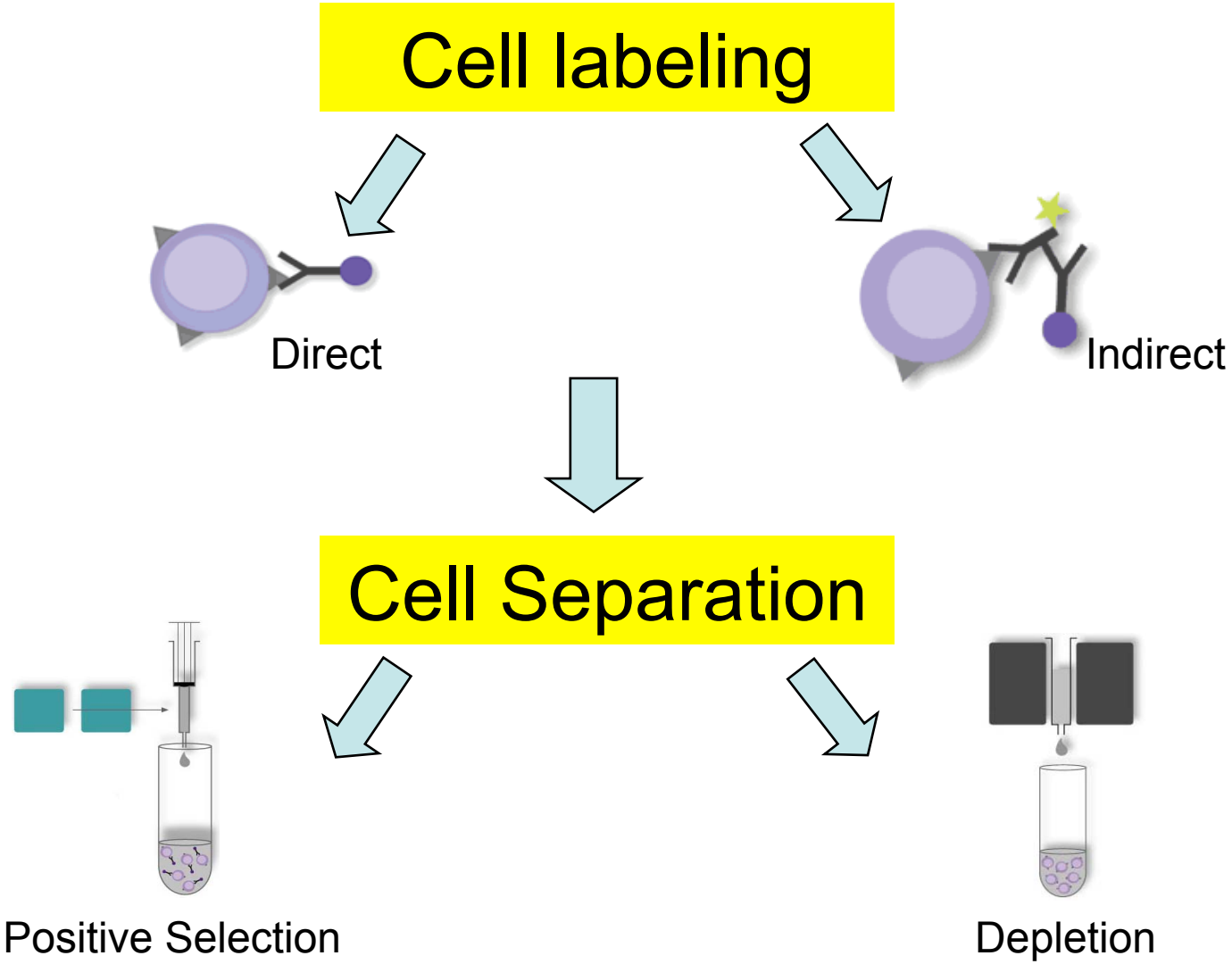
Strategy:



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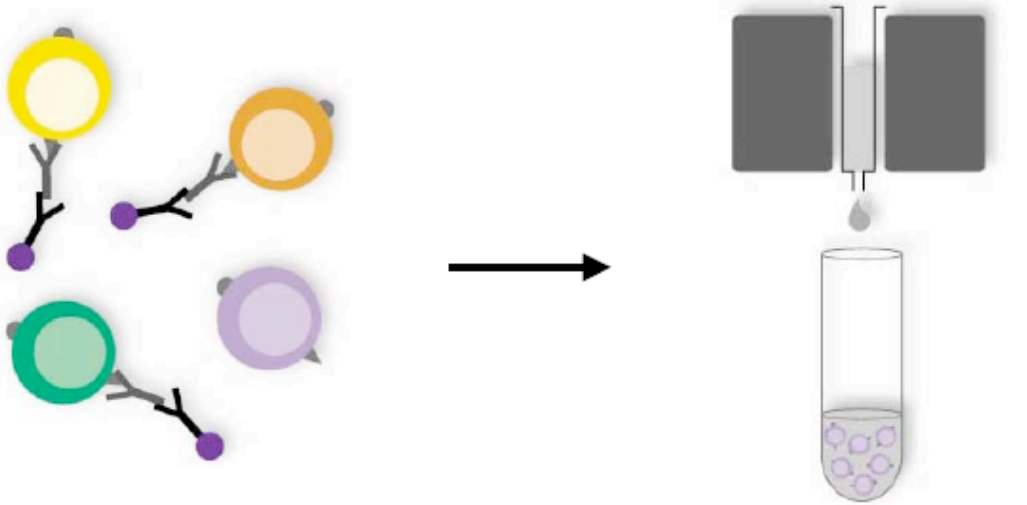
Depletion strategy



Magnetic labeling

Isolation of untouched cells

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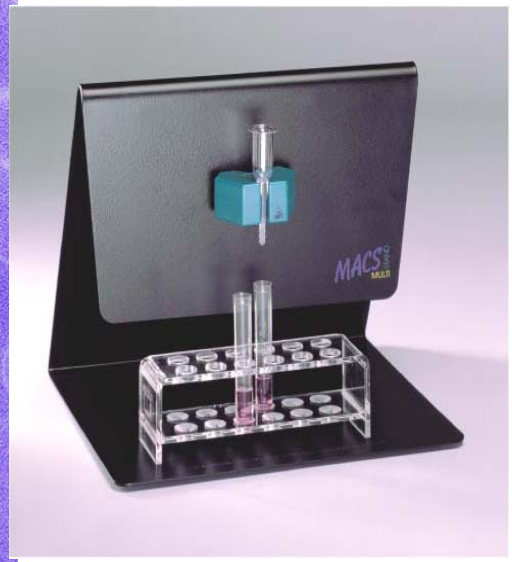
MACS™ Separators

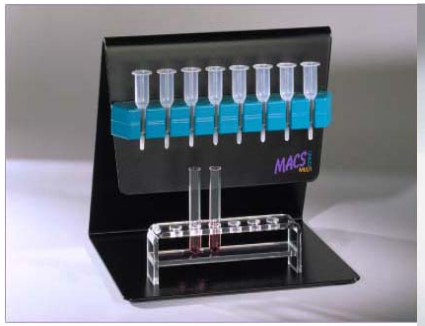
MiniMACS™ Separator



MS Column

autoMACS™ Instrument

for separation of $2x10^8$ total cells or $1x10^7$ labeled cells



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autoMACS™ Pro Separator

Key benefits:

- **Based on renowned MACS® Technology**
  - **Walk-away cell sorting of multiple samples**
  - **Easy operation**
  - **Standardized procedure for reproducible results**

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autoMACS™ Pro Separator

Easy operation

- **Intuitive software**
  - Touchscreen
  - Pre-set separation and rinsing programs
  - Monitoring of buffer and column status, sampler, and cooling tube racks
- **Ready-to-use buffers**
  - autoMACS™ Running Buffer
  - autoMACS™ Pro Washing Solution
  - Buffer bottle illumination
- **Reusable autoMACS™ Columns**
- **Compact benchtop design**
  - Fits in laminar hood

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MACS® Whole Blood MicroBeads

- **Developed for autoMACS™ and autoMACS Pro Magnetic Cell Sorting**
- **Human hematopoietic subsets directly from whole blood**
- **Rapid—no density gradient centrifugation, no erythrocyte lysis**
- **Ideal for molecular studies, e.g., chimerism analysis**
- **Safe handling of hazardous samples, e.g., HIV samples**
- **CD15, CD3, CD4, CD8, CD56, CD19, CD45, CD14 Whole Blood MicroBeads**

  autoMACS™ Separator: small sample volumes from 0.25–3 mL

  autoMACS™ Pro Separator: sample volumes from 0.25–15 mL

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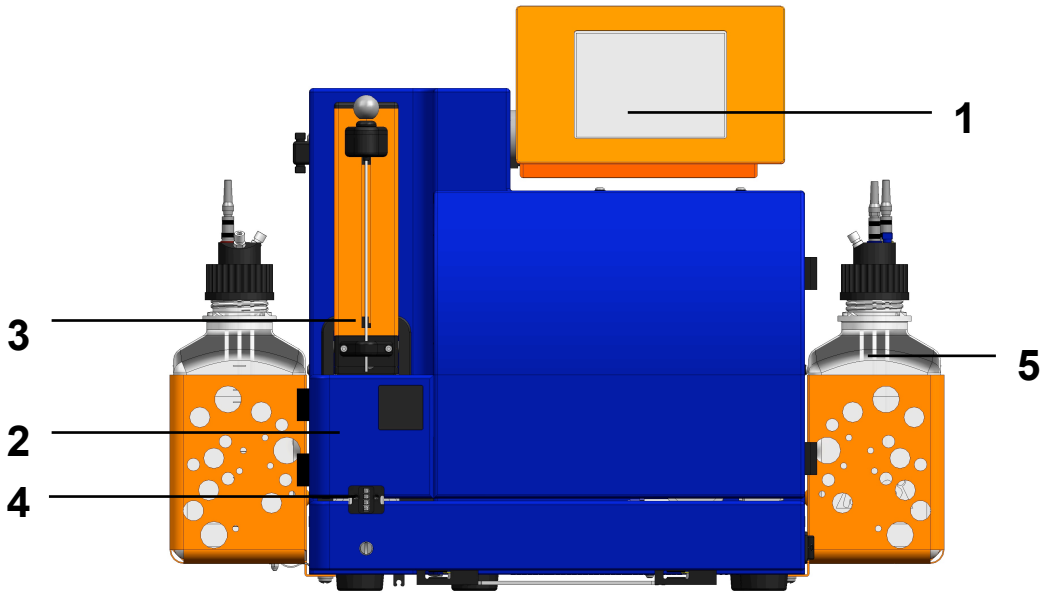
autoMACS™ Pro Separator

Key components (I)

1. Touchscreen
2. Washing station
3. Robotic arm
7. Rack detection
8. Fluid container (4 x)



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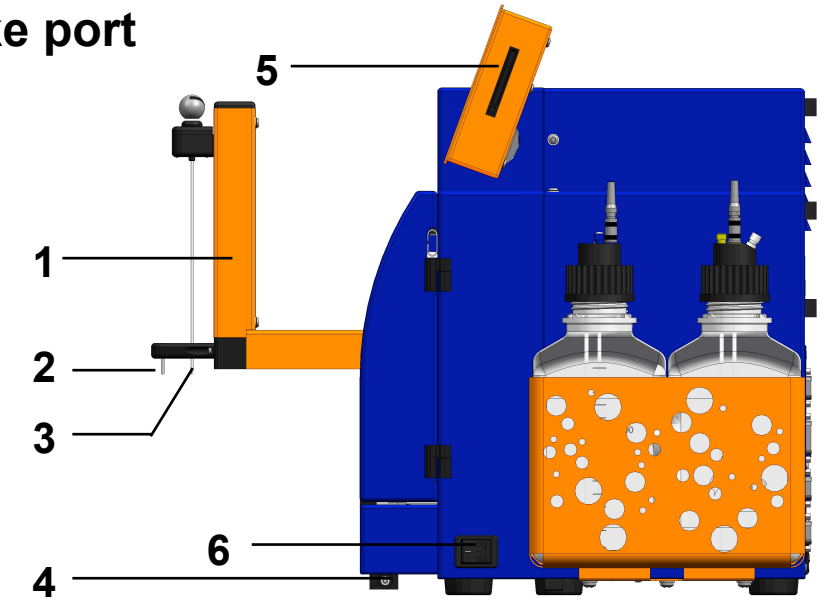
autoMACS™ Pro Separator

Key components (II)

1. Robotic arm
2. Outlet port neg. fraction
3. Outlet port pos. fraction / uptake port
4. MiniSampler guiding
5. Memory card
6. Power switch



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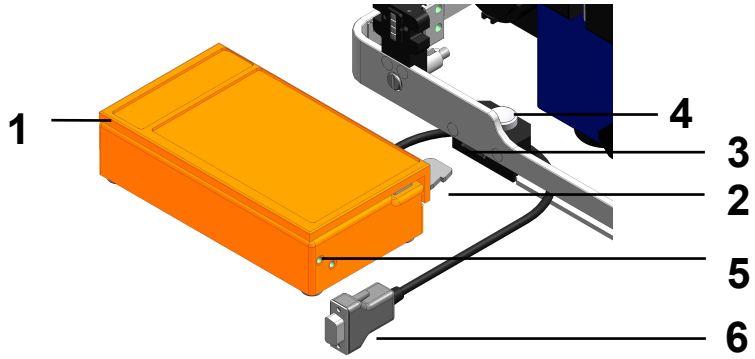
autoMACS™ Pro Separator

MACS® MiniSampler

- **Moves tube rack into x-direction**
- **Connects directly to the autoMACS™ Pro Separator**
- **Easy installation**
- **Lid for maximum sample protection**
- **Automatic detection by autoMACS™ Pro Separator**

1. MACS® MiniSampler
2. MiniSampler guiding
3. Socket for MiniSampler
4. MiniSampler release button
5. MiniSampler lid guiding
6. Plug (connection to autoMACS™ Pro Separator)



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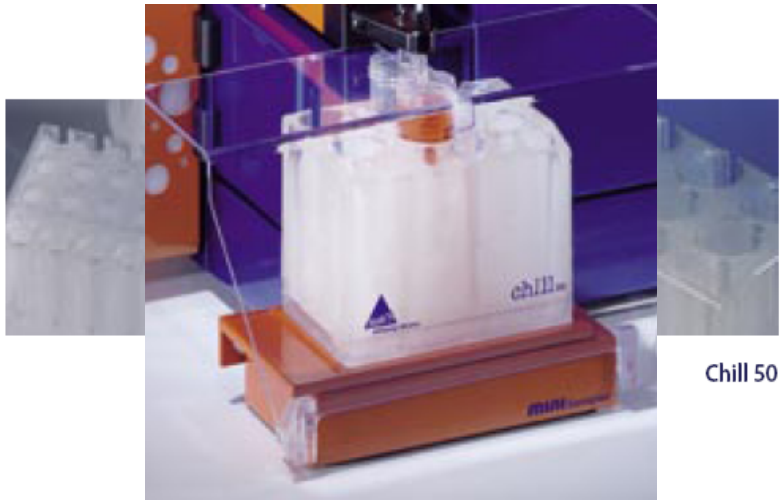
autoMACS™ Pro Separator

MACS® Cooling Tube Racks for optional cooling



Rack type	Max. no. of tubes	Max. vol. per sample	Max. no. of cells
Chill 5	6 (5 mL)	2.5 mL	5×10^8
Chill 15	5 (15 mL)	12.5 mL	2.5×10^9
Chill 50	3 (50 mL)	50 mL	4×10^9

- **Optional cooling to sustain cell viability**
- **Lid for maximum sample protection**
- **Automatic recognition of different tube racks**

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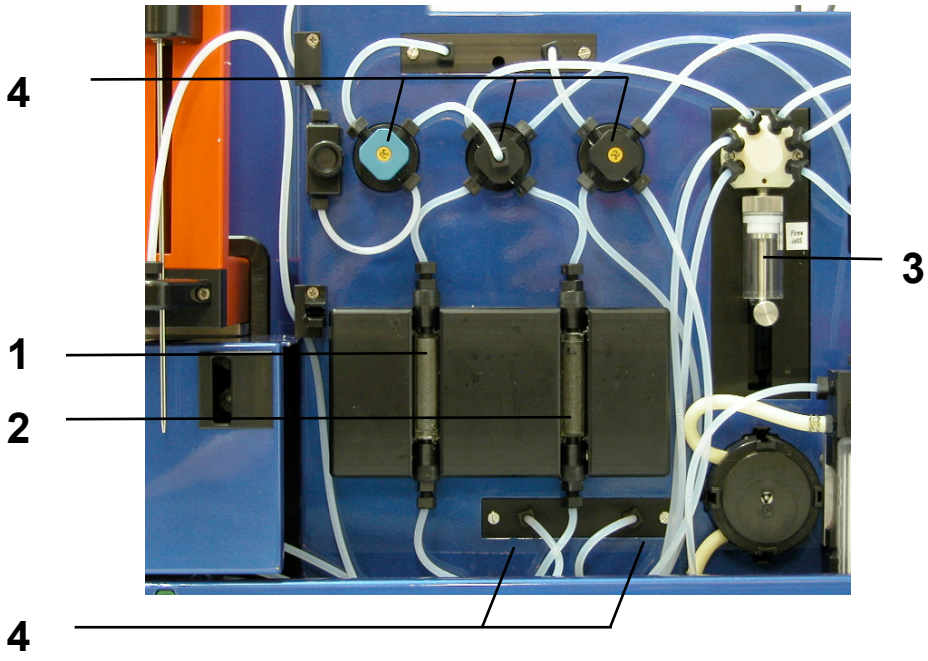
autoMACS™ Pro Separator

Key components (III)

1. Separation column 1
2. Separation column 2
3. Pump
4. Five valves



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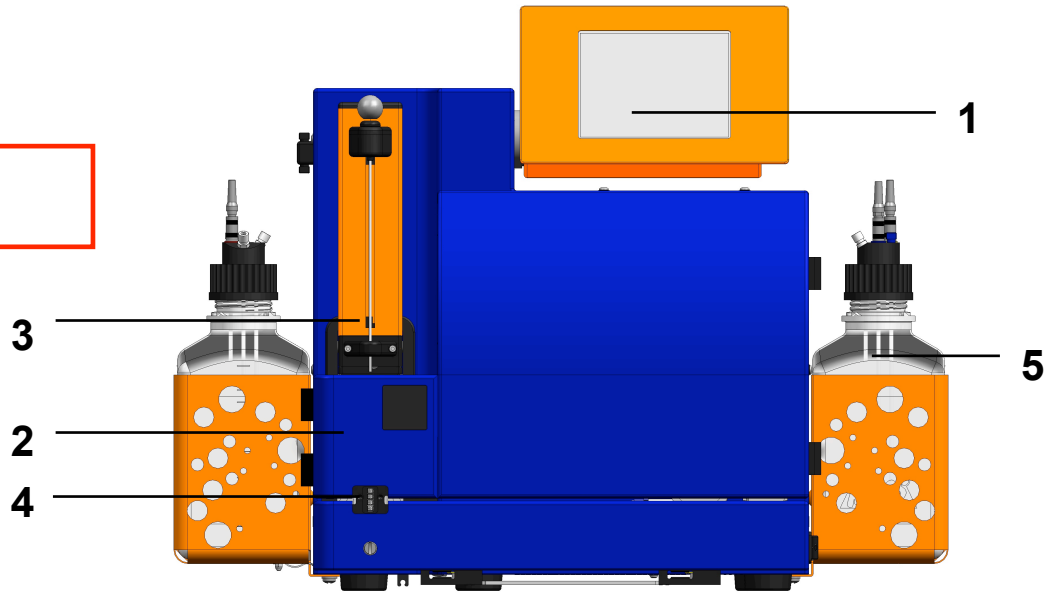
autoMACS™ Pro Separator

Key components (I)

1. Touchscreen
2. Washing station
3. Robotic arm
7. Rack detection
8. Fluid container (4 x)



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autoMACS™ Pro Operation

Fluid containers

1. **Connect fluid sensor cables**
2. **Fill or empty fluid containers accordingly**

Container	Content	Color-code	Order no.	Symbol
autoMACS™ Running Buffer	PBS, 0.5% BSA, EDTA	Blue	**130-091-221**	
autoMACS™ Pro Washing Solution	Salt solution with detergent	Green	**130-092-987**	
Storage Solution (70% ethanol)	100% ethanol, analytical reagent grade, diluted with distilled H_2O	Black	NA	
Waste	Empty	Red	NA	

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autoMACS™ Pro Operation

Fluid containers

Bottle illumination indicates status of the instrument

Code	Status	User action
Green	Ready for separation	No action required
Blue	Instrument operating	No action required
Yellow	Not ready for separation	Run wash program (“Rinse” or “Qrinse”)
Red	Error	Check screen for error correction
Purple	Program “Sleep” is completed	Switch off autoMACS™ Pro Separator
Blinking	Action required	Check screen for required action

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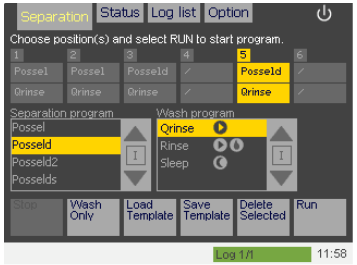
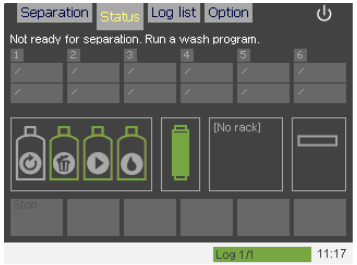
autoMACS™ Pro Separator Software

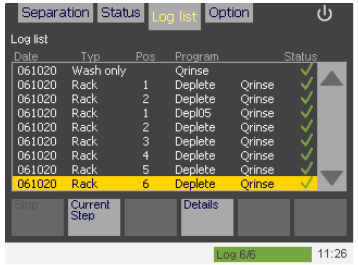
Easy operation with intuitive screen menus

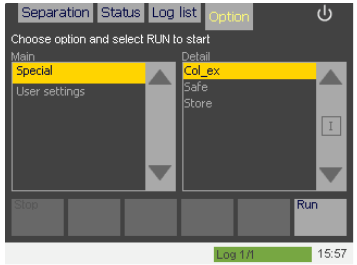
Software dialog menus: overview

- **Separation menu**—to program separation sequences and start the process
- **Status menu**—to monitor instrument status and progress of separation
- **Log list menu**—to access process details and history
- **Option menu**—to access special programs and user settings









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autoMACS™ Pro Operation

Rinsing and cleaning programs

- **Qrinse (Quick Rinse; standard short rinse)**
  - 1.5 min
  - Rinsing and refilling with autoMACS™ Running Buffer
- **Rinse**
  - 4 min
  - Rinsing with autoMACS™ Pro Washing Solution;
    rinsing and refilling with autoMACS™ Running Buffer
  - For priming of the autoMACS™ Pro Separator
  - Recommended before isolation of rare cells and between whole blood separations

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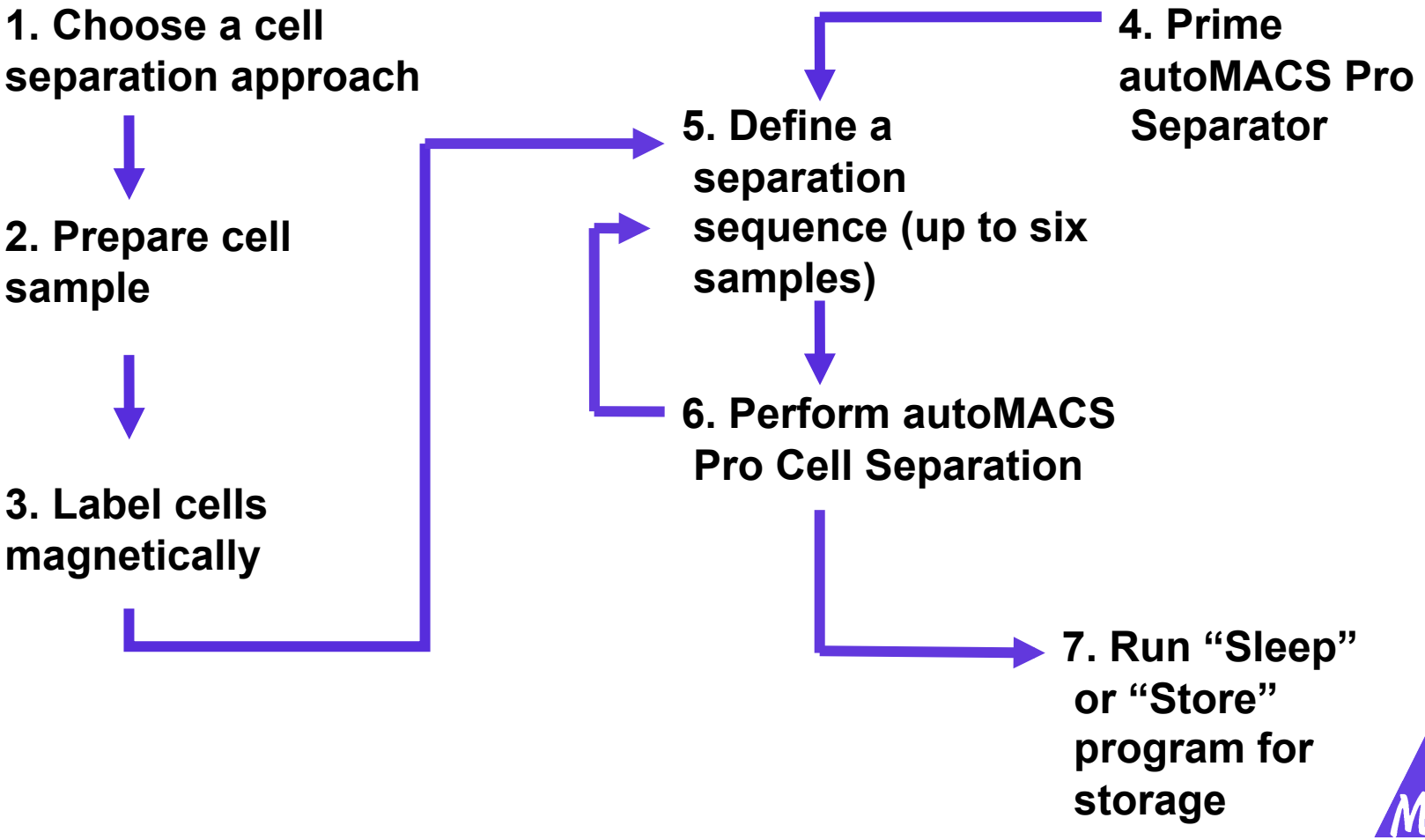
autoMACS™ Pro Separator

How to work with the instrument



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autoMACS™ Pro Operation

Choose a separation program

Positive selection versus depletion
Single-column versus double-column selection
Standard mode versus sensitive mode

Goal		Obtain a cell population expressing a particular cell surface antigen		Eliminate cell subpopulation(s) from your cell sample and obtain “untouched” cells
Strategy		**Positive selection** Magnetically label the target cells		**Depletion** Magnetically label cells other than the target cells
		Cells with normal to high frequency	Rare cells, or increase of purity	
Program	Normal to high antigen expression	**POSSEL** Positive selection	**POSSELD** Double positive selection	**DEPLETE** Depletion
	Low antigen expression	**POSSEL_S** Sensitive positive selection	**POSSELD2** Also for small blood volumes	**DEPLETES** Sensitive depletion
		NEW !	**POSSELWB** For blood volumes from 3 mL to 15 mL	**DEPL05/025** Special depletion
			POSSELDS Sensitive double-positive selection	

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autoMACS™ Pro Operation

Pre-set separation programs

Positive Selection	Single- or double-column program	Antigen expression	Target cell frequency	Sample loading rate
Possel	Single	Normal	>5%	4 mL/min
Possels	Single	Low	>5%	1 mL/min
Posseld	Double	Normal to low	Rare, <5%	4 mL/min
Posseld2	Double	Normal	Rare, <5% 0.25 mL–3 mL whole blood	4 mL/min
Posselwb	Double	Normal	Rare, <5% 0.25–15 mL whole blood	4 mL/min
Posselds	Double	Low	<5%	1 mL/min

Depletion	Single- or double-column program	Antigen expression	Depletion efficiency	Sample loading rate
Deplete	Single	Normal	Depletion efficiency increases with lower sample loading rate	4 mL/min
Depletes	Single	Low		1 mL/min
Deplete05	Single	Low		0.5 mL/min
Deplete025	Single	Low		0.25 mL/min

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autoMACS™ Pro Maintenance

Programs

- **Programs**
  - **Sleep**
    - Rinsing with autoMACS™ Pro Washing Solution; rinsing and refilling with Storage Solution
    - Before instrument is switched off for overnight storage
  - **Safe**
    - Recommended for decontamination with hypochlorite solution
  - **Store**
    - Prior to storage period longer than 2 weeks
    - Substitute columns should be installed
  - **Column exchange**
    - Every 2 weeks

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Standardized procedure for reliable results

Examples

Separation results with autoMACS™ Magnetic Cell Sorting

- Positive selection and depletion
- From rare to frequent cells
- From PBMCs, whole blood, tissue
- From any species

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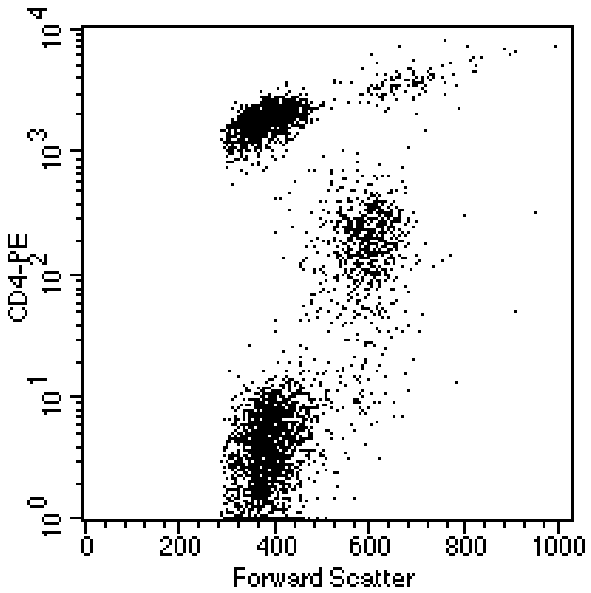
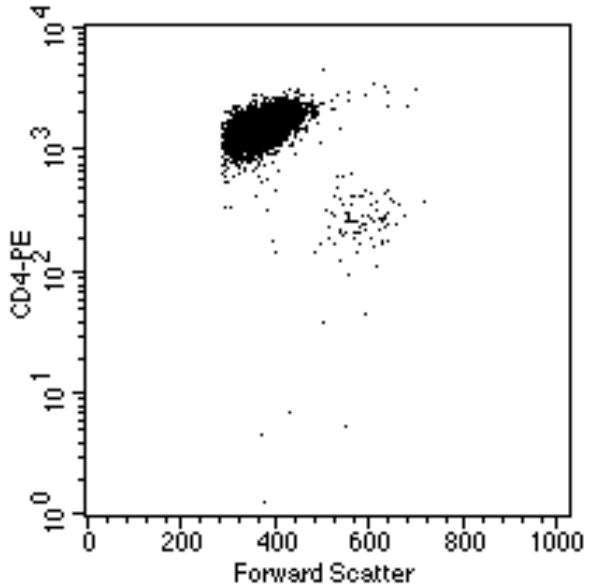
Positive selection from PBMCs

CD4 MicroBeads, human





Source: PBMCs
Program: Possel
Purity: >98%

MACS

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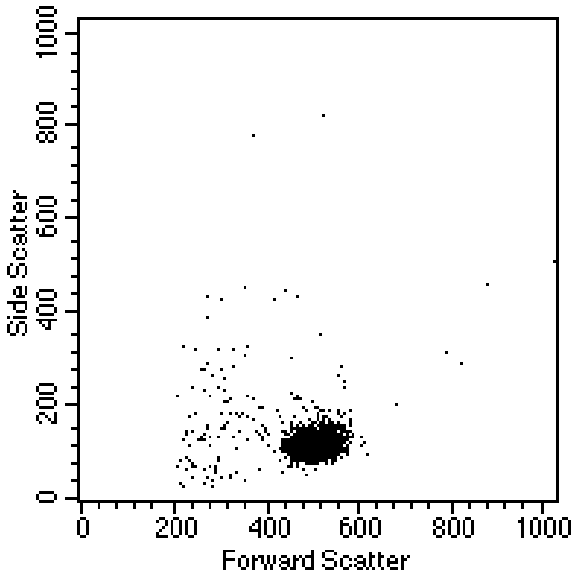
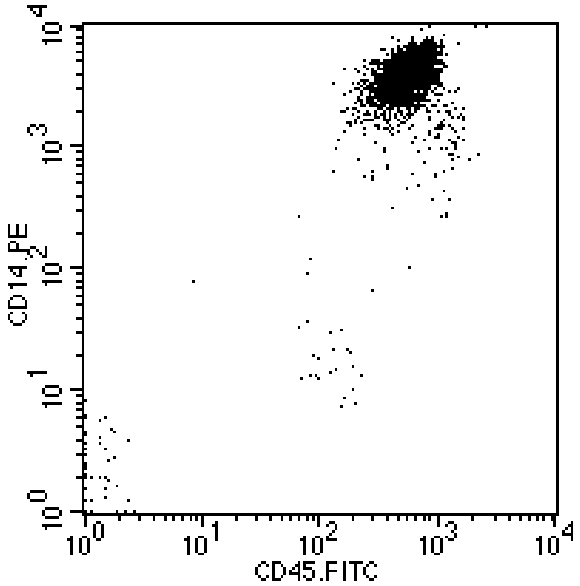
Positive selection from whole blood

Monocyte isolation with Whole Blood CD14 MicroBeads





Source: Anti-coagulated whole blood
Program: Posseld2
Purity: >98%

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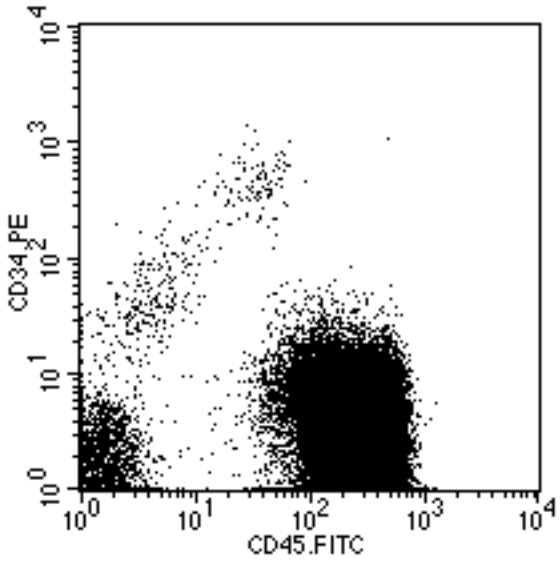
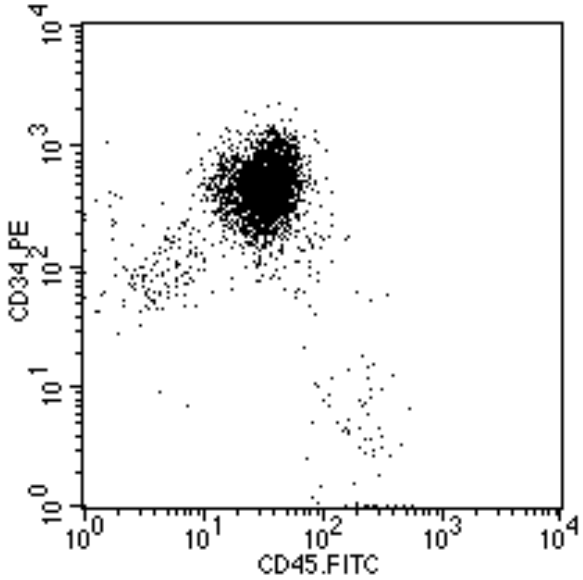
CD34+ stem cell isolation from PBMCs

CD34 MicroBead Kit, human





Source: PBMCs
Program: PosseId2
Purity: >91%

MACS

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Summary

- Walk-away processing of up to six samples
- Automated sample uptake, fraction elution, system cleaning
- Sample cooling option
- Safe sensor-controlled process
- Optimized programs for a wide variety of cell separations
- Based on renowned MACS® Technology
- Compact benchtop design, also fits in laminar flow hood

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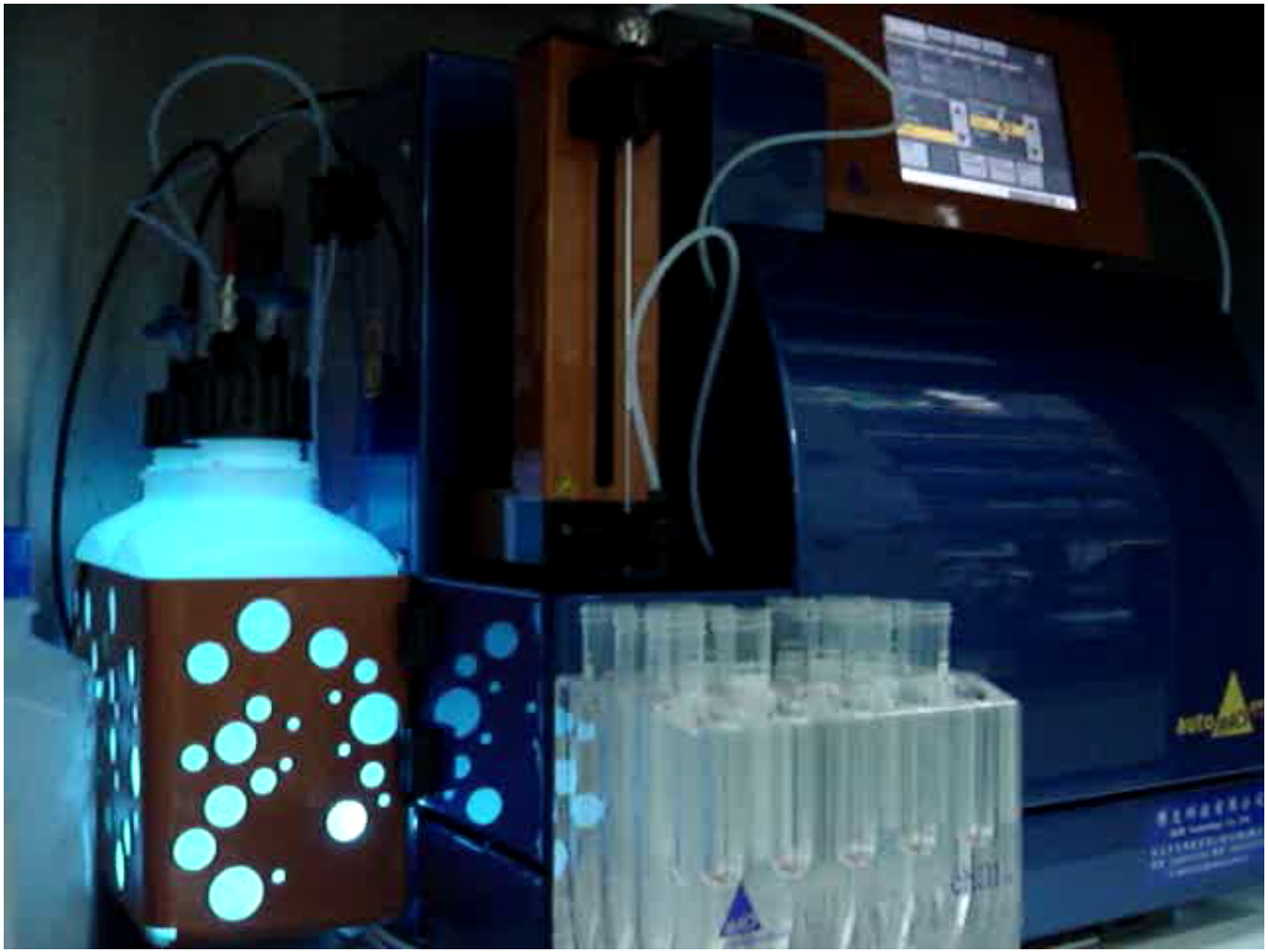

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可應用於...

無菌細胞培養、Flow分析、
以及 分生實驗!

Easy & Safe

- 針對常用組織，內建最佳化均質條件 -- 標準操作，結果穩定
- 無需灌流
- 不用再剪組織剪到手軟
- 封閉式無菌樣品管設計
  --減少汙染、增加安全性

Gentle & Effective

- 實驗證明，比傳統手動方式獲得更多存活脾臟細胞。
- 可同時處理多顆脾臟
- 一分鐘完成組織均質
- 兩小時內得到單一細胞

成功案例

> mouse splenocytes 分離
> mouse liver cells 分離
> mouse neural tissue single cell suspension分離
> Tissue中 total RNA/mRNA萃取
> and so on... you can be the next one.

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Thank you for your attention!

Evelyn Lin

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