MACS
Miltenyi Biotec

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MACS Technology

autoMACS Pro™ Cell Separator

J & H 博克科技有限公司

產品應用專員 曾筱筑

Application Specialist *Sarah Tseng*

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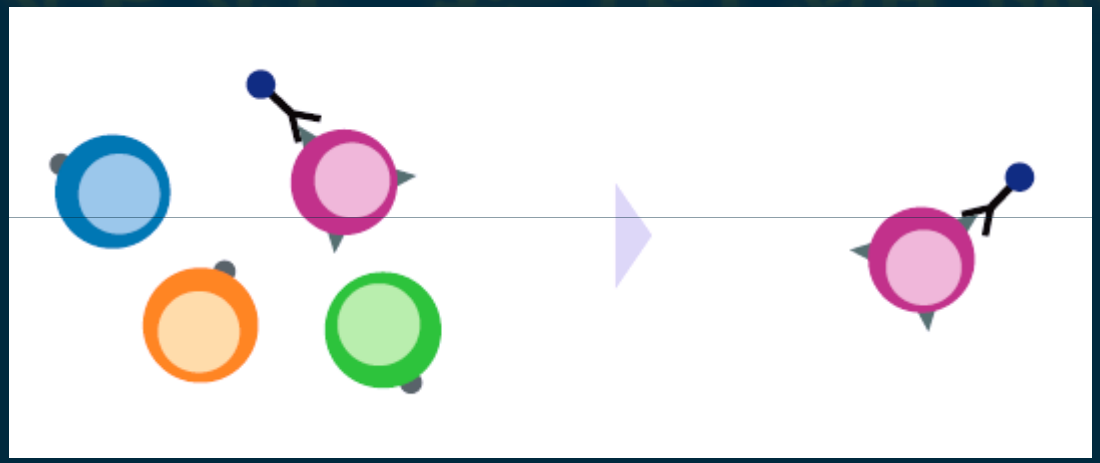
2. Procedure of Magnetic Cell Separation

3. autoMACS Pro Introduction

MACS™-Magnetic Activated Cell Sorting

抓住你要的細胞!!



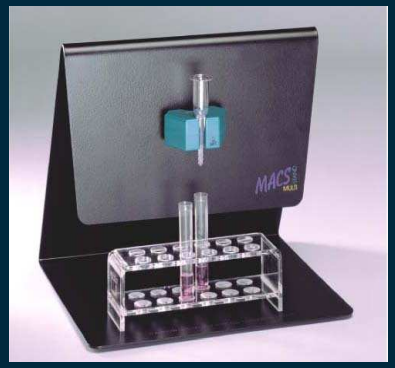
MACS
Miltenyi Biotec

Germany

- Fundation in 1989
- The gold standard for cell separation.
- More than 12,000 publication in 20 years

MACS™ Separation systems

MiniMACS™ Separator



For separation of $1x10^7$ labeled cells

MidiMACS™ Separator

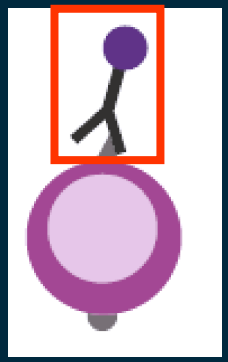
For separation of $1x10^8$ labeled cells

autoMACS™ Pro Separator

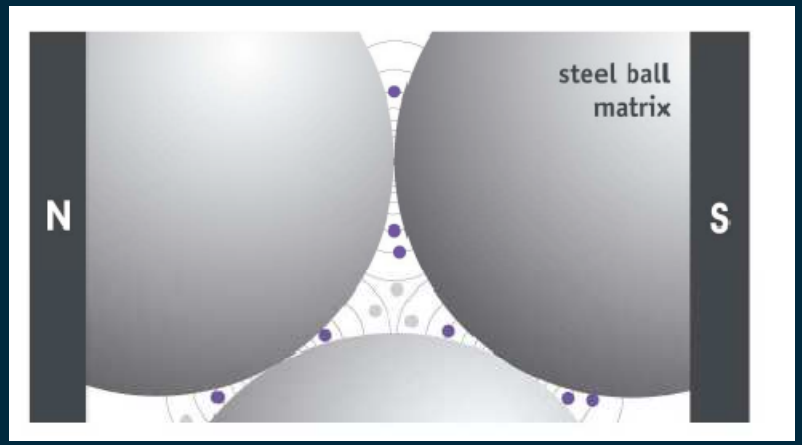
-- Walk-away cell sorting of multiple samples
-- >10^7 cells/ sec
-- Reusable Column
-- Stem cell separation from whole blood

MACS™-Magnetic Activated Cell Sorting

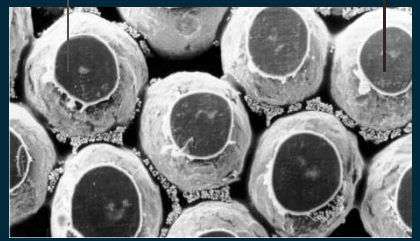
MicroBeads



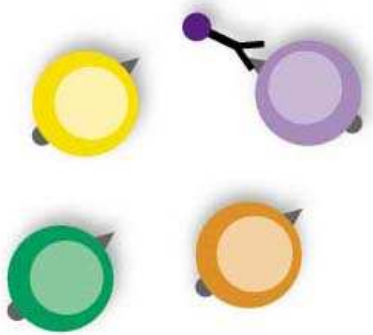
Columns



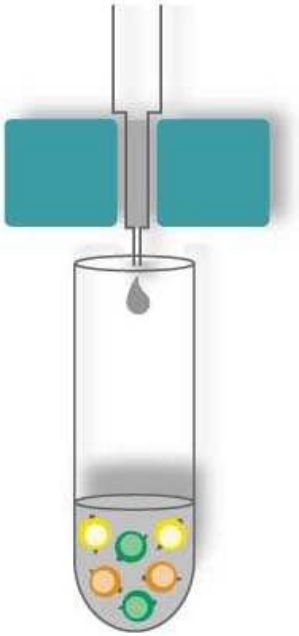
Separators



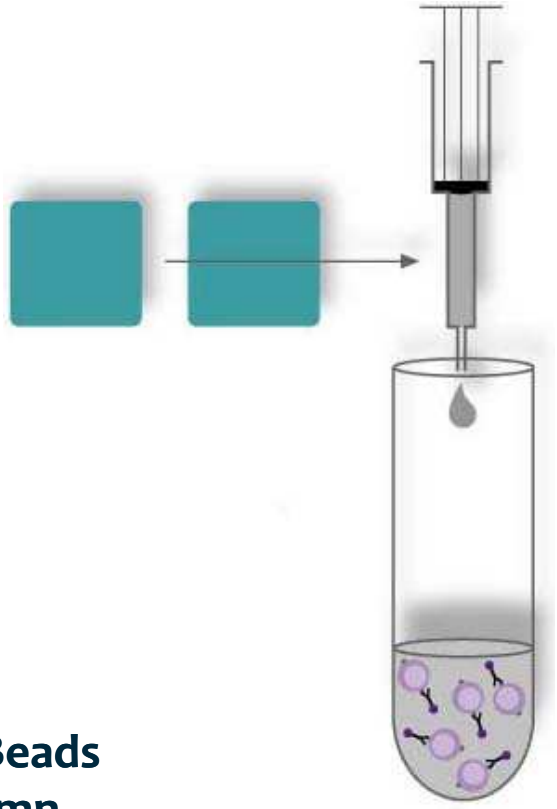
Based on renowned MACS™ Technology



Magnetic labeling with MACS® MicroBeads

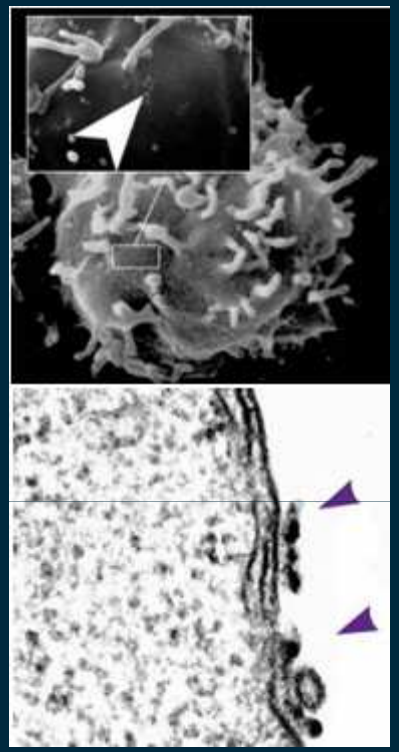


Cells labeled with MACS® MicroBeads are retained in the MACS® Column. Unlabeled cells pass through and are collected as the negative fraction



Elution of the labeled cell fraction

Questions about microbead…



- Does microbead activate my target cell?

  → **Small beads: 50 nm**

  → **Iron Oxide and Polysaccharide**

- Is it necessary to remove the microbeads?

  → **Non-toxic and biodegradable**

- Does it affect the fluorescent antibody binding?

  → **Only 20-30% of binding sites were occupied**

- **Highly specific** -- Ideal for selection of rare cells
- **Colloidal suspension** – prevent precipatation and aggregation

Features and Benefits of MACS

- Fast and easy
- Highly pure cells(＞95%)
- Highly recoveries from normal donors (＞90%)
- Reproducibility
- Cell function preserved

Contents

1. MACS Technology

2. Procedure of Magnetic Cell Separation

3. autoMACS Pro Introduction & Software overview

Procedures of MACS cell separation

Sample preparation –
Obtain single cell suspension

↓

Labeling with MACS microbeads

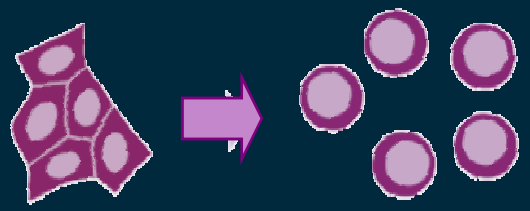
↓

Cell separation

Sample preparation

- From Peripheral Blood
  Density-gradient Centrifugation
  RBC lysis

- From tissue and organs



gentleMACS™Dissociator

The safe, gentle and easy way of automated tissue dissociation

For obtaining…
Single cells from
> mouse spleen, neural tissue
> mouse heart, lung, liver
> Tumor tissue
> Any other tissue sample
Total RNA/mRNA from tissues

Procedures of MACS cell separation

30µm pre-separation filter

Sample preparation –
Obtain single cell suspension

↓

Labeling with MACS microbeads

↓

Cell separation

Labeling with MACS microbeads

General Way…

What kind of cell you want?
"I want mouse T cells"

↓

Specific Markers?
"They expresses CD3"

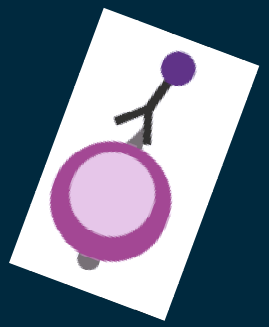
↓

Does Miltenyi Biotec provide specific Microbead?
"yes! They provide mouse CD3 microbead!"

↓

Stain with the specific Microbead…

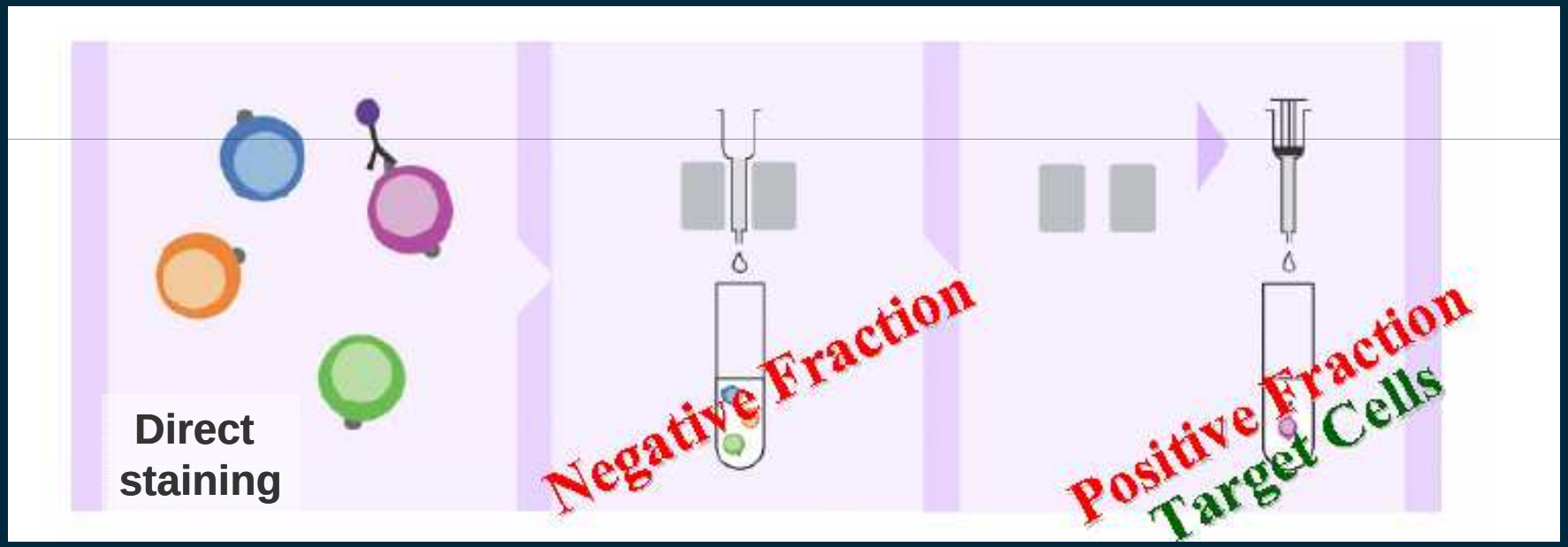
(Usually 4-8℃, 15mins)



Labeling with MACS microbeads

General Way…

Positive Selection



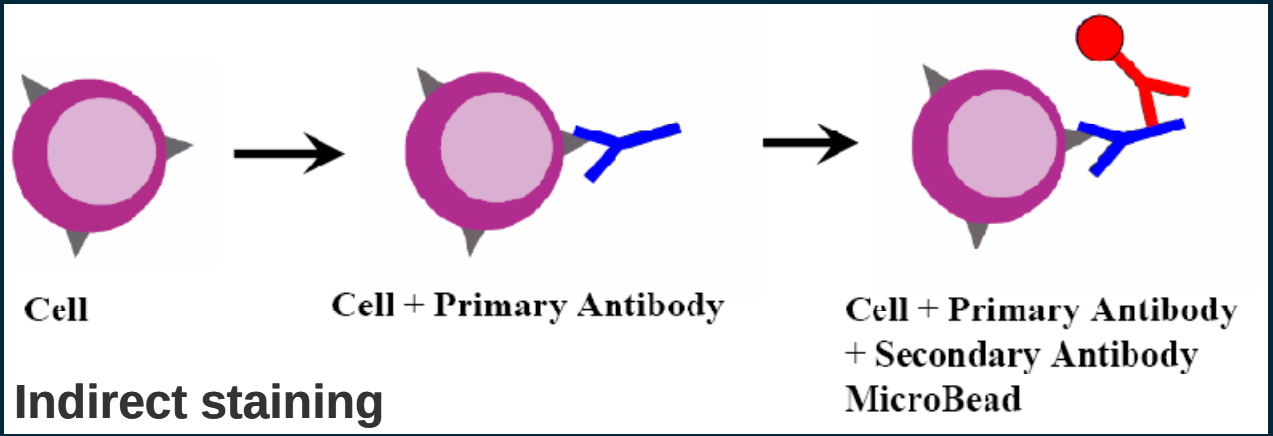
More than 200 direct microbead reagents

Labeling with MACS microbeads

What if…

" There is no direct microbeads for my specific marker?"

Indirect Microbeads Labeling!!



Indirect Microbeads :

- ✓ anti-Immunoglobulin microbeads (IgG、IgM…)
- ✓ anti-Fluorochrome microbeads (FITC、PE、APC…)
- ✓ Streptavidin/anti-biotin microbeads

Labeling with MACS microbeads

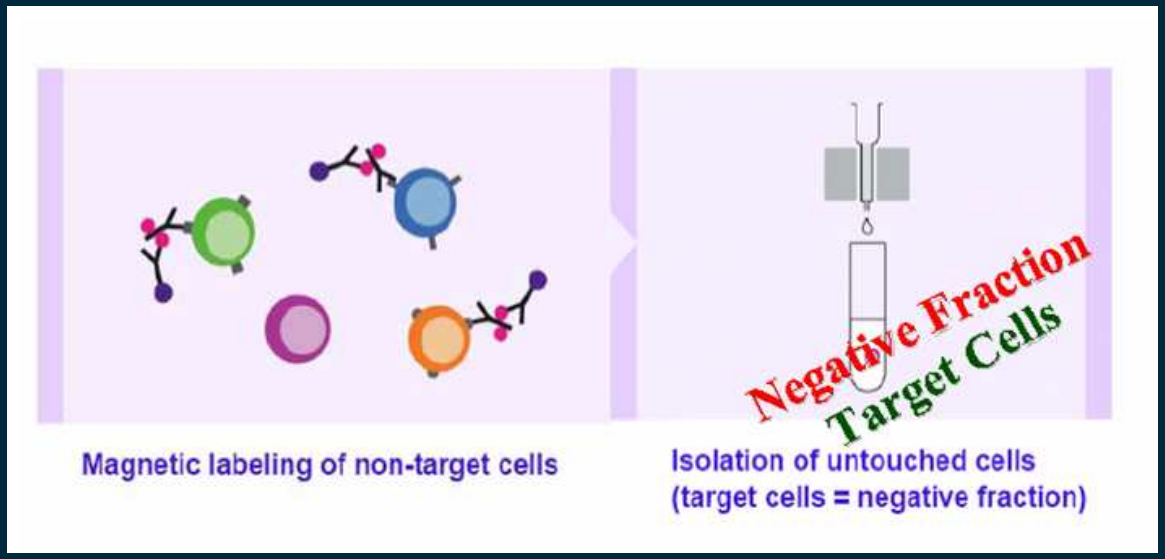
What if…

“ I want to deplete specific cell population.”

“ I don’t want my cells to be bound with any antibodies!!”

Depletion / Untouched separation

(Label cells which you don’t want!)



CD3 MicroBeads, human (positive selection) V.S Pan T cell isolation kit (Depletion)

Labeling with MACS microbeads

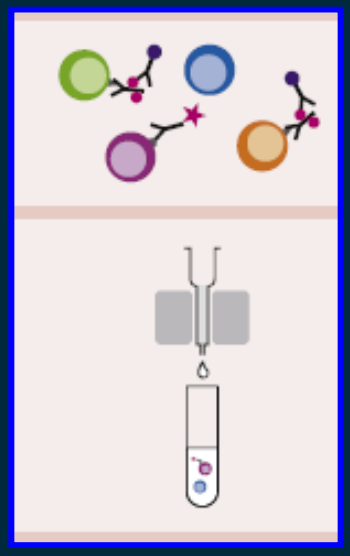
What if…

" I want to sort cells with more than one marker?"

1. Depletion→Positive selection

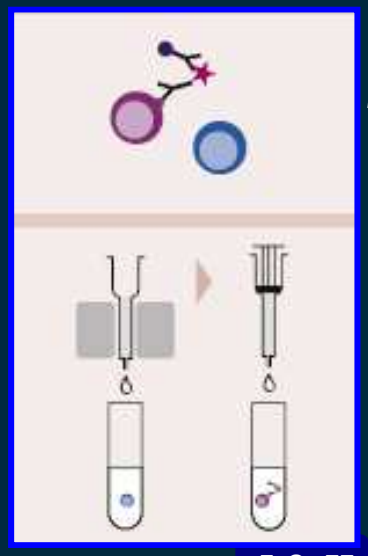
For example **:**

Sorting CD4+CD25+ cells from PBMC<Regulatory T cell isolation kit>



Magnetic labeling of non-CD4 cells

Depletion of non-CD4 cells



Magnetic labeling of CD25+ cells

Positive selection of CD25+ cells

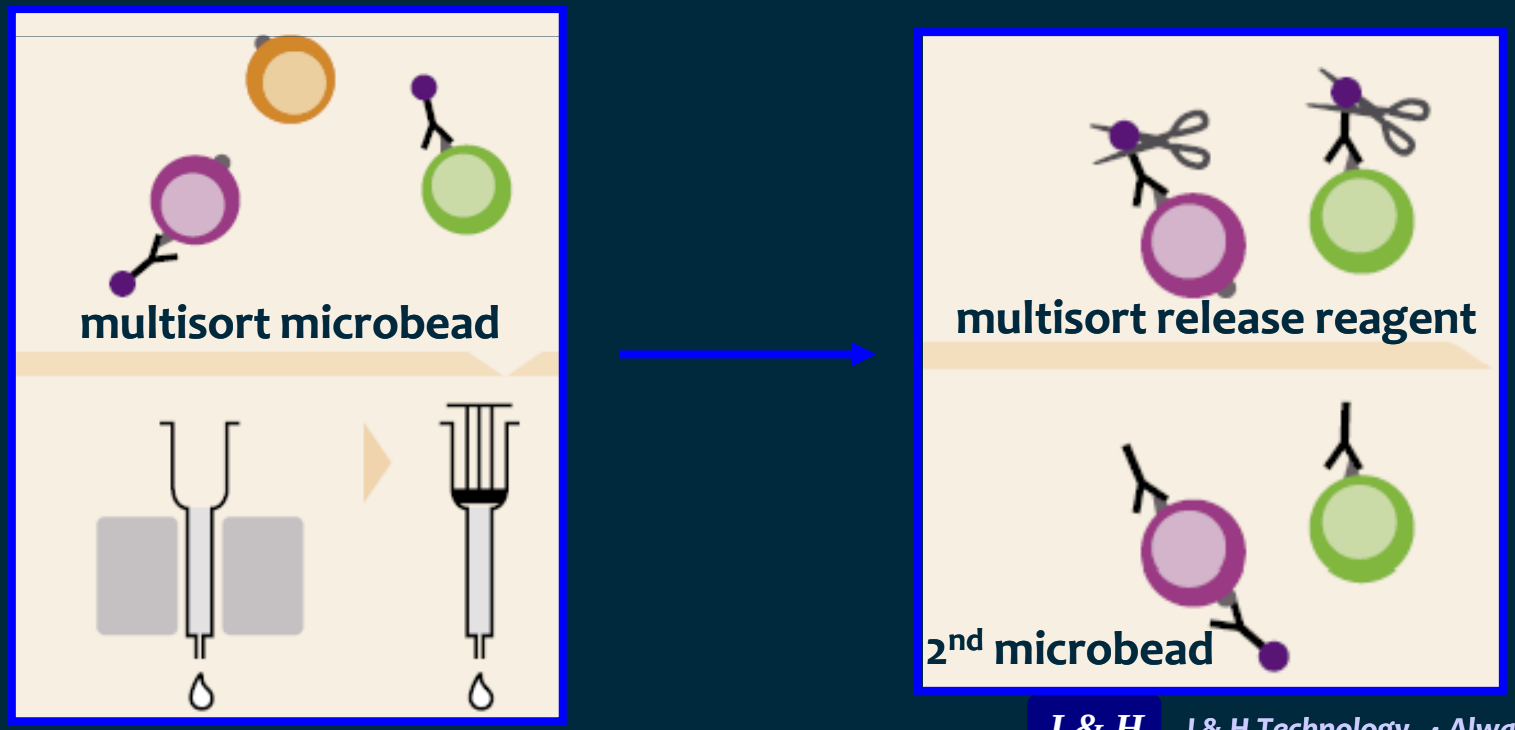
Labeling with MACS microbeads

What if…

“ I want to sort cells with more than one markers?”

2. Multisort Separation

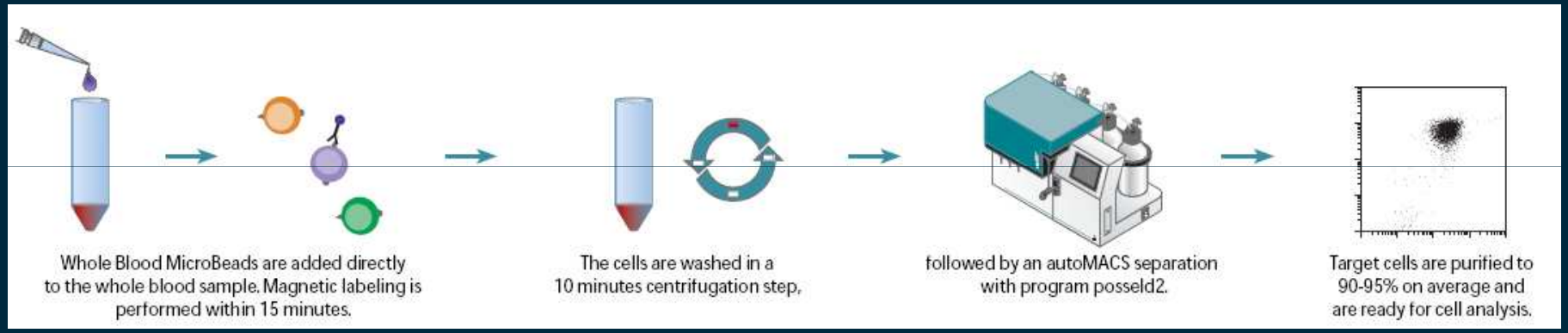
<<MACS Multisort kits>>



Labeling with MACS microbeads

Cell sorting from whole blood (0.25 mL–3 mL)

<<MACS Whole blood microbeads>>



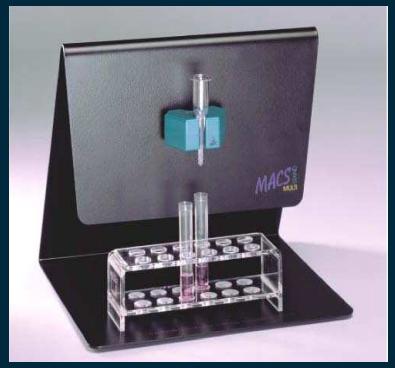
MACS® Whole Blood products

Whole Blood CD3 MicroBeads	Whole Blood CD15 MicroBeads
Whole Blood CD4 MicroBeads	Whole Blood CD19 MicroBeads
Whole Blood CD8 MicroBeads	Whole Blood CD45 MicroBeads
Whole Blood CD14 MicroBeads	Whole Blood CD56 MicroBeads

Contents

MACS™ Separation systems

MiniMACS™ Separator



For separation of $1x10^7$ labeled cells

MidiMACS™ Separator

For separation of $1x10^8$ labeled cells

autoMACS™ Pro Separator

autoMACS™ Pro Separator

Walk-away cell sorter for multiple samples

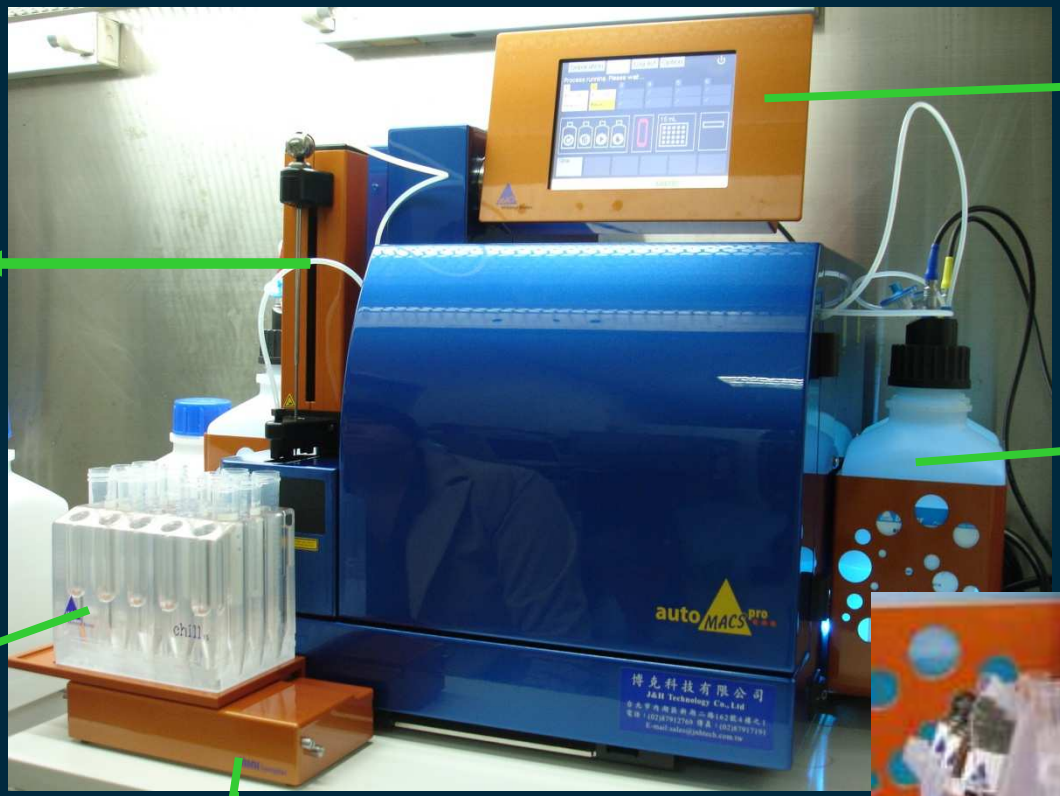
- **Fully Automated**
  Cleaning, Sample uptake, Separation
- **Automated microbead labeling**
- **>10^7 cells/ sec**
- **Processing of up to 6 samples**
- **Easy-to-use software**
- **Sensor-controlled process**
  buffer fluid levels, column status, sample rack type,
  Bottle illumination indicates instrument status.
- **Simple three steps:**
  1. Scan reagents using barcode readerd
  2. Position samples and reagents
  3. Select the separation strategies

  …Just walk away and have your tea time~

autoMACS™ Pro Separator

autoMACS™ Pro Separator

Walk-away cell sorting of multiple samples

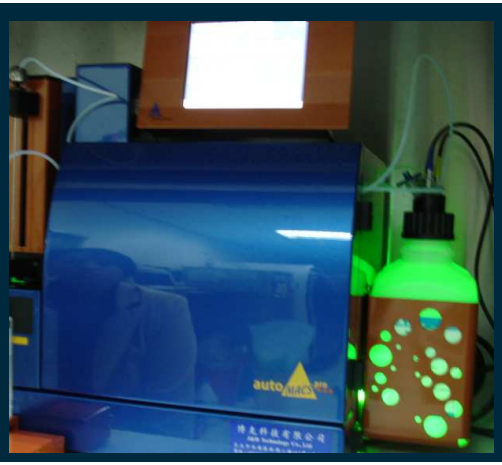


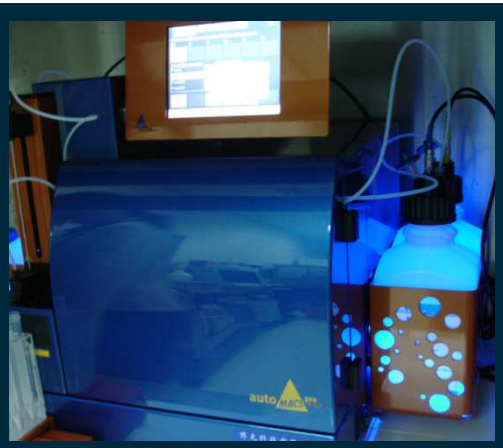
autoMACS™ Pro Separator

Walk-away cell sorting of multiple samples

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
Red	Error	Check screen for error correction
Purple	"Sleep" is completed	Switch off autoMACS™ Pro Separator
Blinking	Action required	Check screen for required action





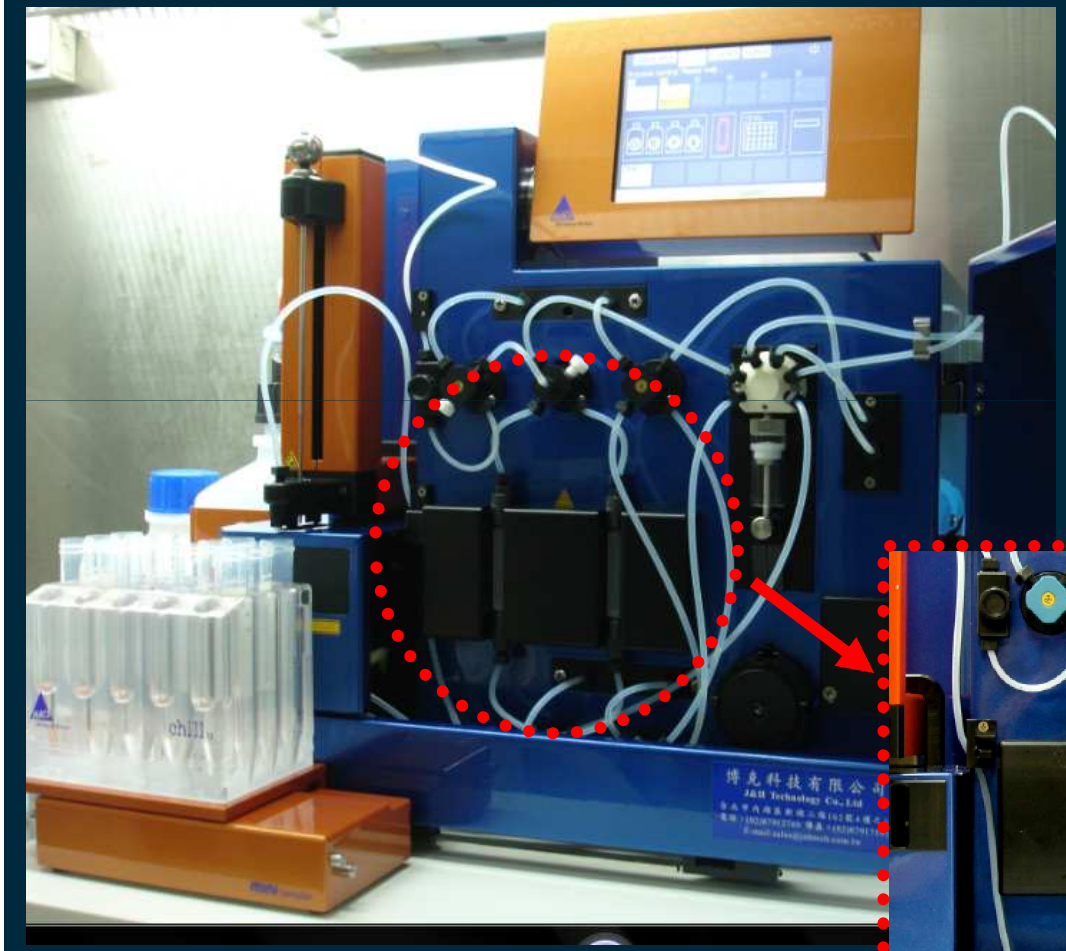
Separation Programs

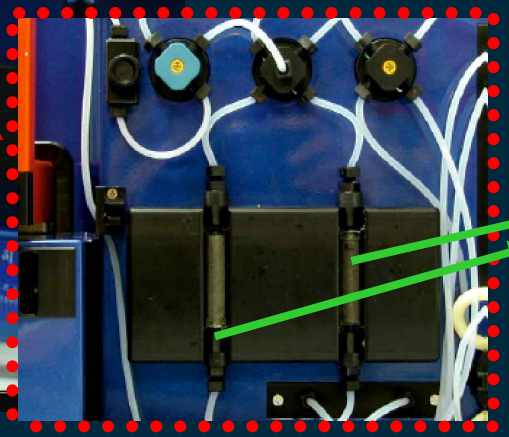
- ✓ Positive V.S Depletion
- ✓ 1 Column V.S double column selection
- ✓ Standard mode V.S sensitive mode(Flow rate)

Objective		Obtain a cell population expressing a particular cell surface antigen		Eliminate cell subset(s) from Sample, obtain 'untouched' cells
Strategy		POSITIVE SELECTION Labeling of target cells		DEPLETION Labeling of non- target cells
		Normal to high frequency (>5%)	Rare cells (<5%), or purity increase	
Program	Normal to high antigen expression	POSSEL Positive selection	POSSELD Double positive selection	DEPLETE Depletion
	Low antigen expression	POSSEL_S Sensitive positive selection	POSSELDS Sensitive double positive selection	DEPLETES Sensitive depletion
	Special programs		POSSELD2 Double positive selec-tion from cord blood, small blood volumes	DEPL05/025 Special sensitive depletion
		NEW !	POSSELWB For blood volumes From 0.25–15 mL	A_Depl7 / A_Depls7 Large scale depletion Up to $1.5x10^9$ labeled cells

autoMACS™ Pro Separator

Walk-away cell sorting of multiple samples





Reusable Separation Columns x2

<< 100 times or 14 days >>

Rinsing and Overnight storage

- **Qrinse (Quick Rinse; standard short rinse)**
  - 1.5 min
  - Rinse and refill with Running buffer
  - Between standard separations
- **Rinse (Intense Rinsing)**
  - 4 min
  - Rinse with autoMACS Pro Washing solution; refill with Running buffer
  - Priming, before rare cell sorting, between blood / bone marrow samples
- **Sleep (Overnight storage)**
  - 5 min
  - Rinsing of fluid system; refill with Storage solution

autoMACS™ Pro Separator

How to work with the instrument

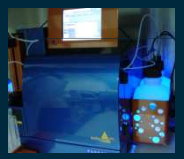
1. Choose a cell separation approach

2. Prepare cell sample

3. Label cells magnetically

4. Prime autoMACS Pro Separator

5. Define labeling and separation sequence (up to six samples)



6. Perform autoMACS Pro Cell Separation

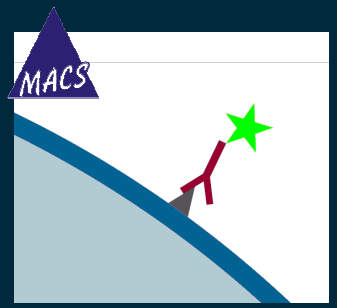
7. Run "Sleep" program and shut down autoMACS Pro.

Downstream Experiments

- **Cell Culture**

Cell culture medium
Cytokines & Growth factor

- **Cell Analysis**



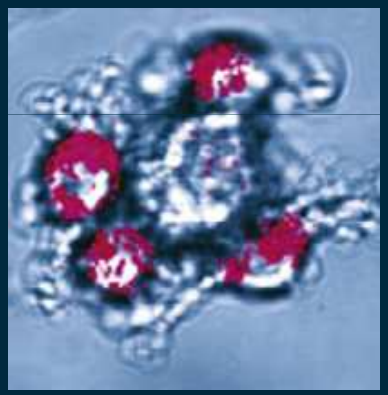
High Quality Antibodies
for detecting various of cells

- **Molecular Biology Research**

Application

Stem cell Research

- **Hematopoietic stem cells / Cancer Stem cells**
  - → **Enrichment :** CD34、CD133 、CD117 microbead kit…



Isolated CD133 cells during cultivation.
Cells were stained with CD133/1(AC133)-PE

Brain tumors

70. Singh, S.K. *et al.* (2003) Cancer Res. 63: 5821–5828. [4254]
71. Singh, S.K. *et al.* (2004) Nature 432: 396–401. [4355]
72. Singh, S.K. *et al.* (2004) Oncogene 23: 7267–7273. [9084]
73. Taylor, M.D. *et al.* (2005) Cancer Cell 8: 323–335. [9024]

Prostate cancer

74. Collins, A.T. *et al.* (2005) Cancer Res. 65: 10946–10951. [5322]

Renal tumors

75. Florek, M. *et al.* (2005) Cell Tissue Res. 319: 15–26. [8267]

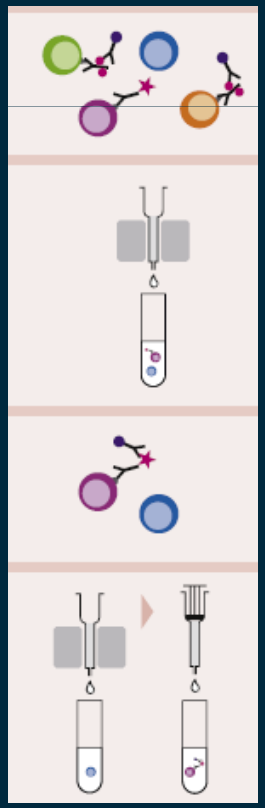
Colon cancer

76. O'Brien, C.A. *et al.* (2007) Nature 445: 106–110.
77. Ricci-Vittiani, L. *et al.* (2007) Nature 445: 111–115.

Application

Immunology

- **T cells**
  - → Total T cells、T cell subsets
  - → Naïve T cells、Activated T cells、Effector T cells、Memory T cells
  - → Regulatory T cell isolation kit ($CD4^+CD25^+CD127^{dim/-}$)、($CD4^+CD25^+$)

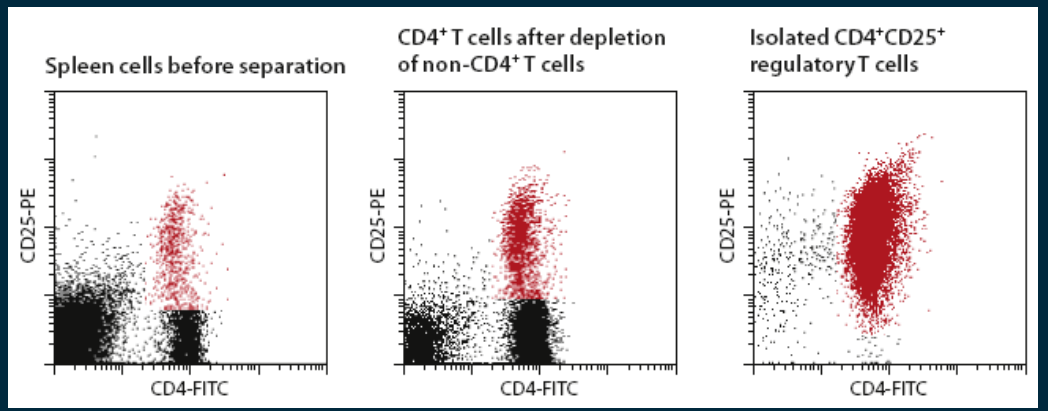


Magnetic labeling of non-CD4 cells

Depletion of non-CD4 cells

Magnetic labeling of non-CD25 cells

Positive selection of CD4CD25 Regulatory T cells



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2. Schwarz *et al.* (2004) J. Immunol. 172: 1036-1043. [3791]
3. Gangi *et al.* (2005) J. Immunol. 174: 7006-7013. [7184]
4. Turk *et al.* (2004) J. Ex. Med. 200(6): 771-782. [6334]
5. Kashiwada *et al.* (2006) J. Immunol. 176: 3958-3965. [8594]
6. Choileain *et al.* (2006) J. Immunol. 176: 225-236. [9411]

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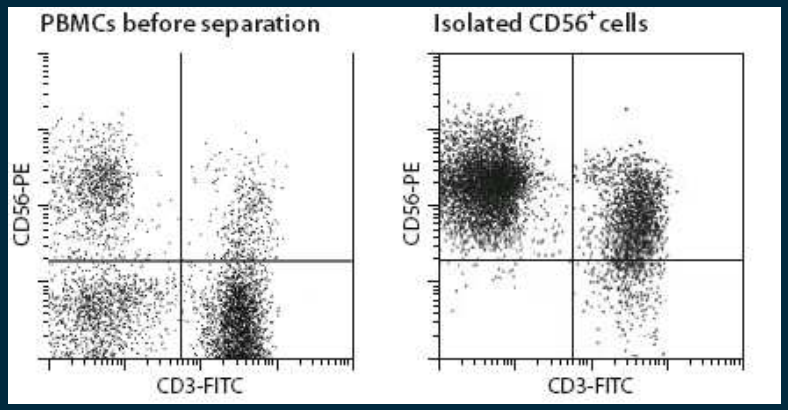
Application

Immunology

- **B cells and B cell subsets**
- **Monocytes**
  → CD11b(MAC-1) 、CD14、CD16、Slan、Monocytes Isolation kit
- **NK cells**
  → CD56、CD49b(DX5)、NK cell isolation kit



Separation of CD56+ NK cells from human PBMC using CD56 microbeads

References

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2. Sinanan, A. *et al.* (2004) Biotechnol. Appl. Biochem. 40: 25–34.
3. De Luna, N. *et al.* (2006) J. Biol. Chem. 281: 17092–17098.
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5. Fletcher *et al.* (1998) J. Immunol. 161: 2365–2374. [558]
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Thank you for your attention !