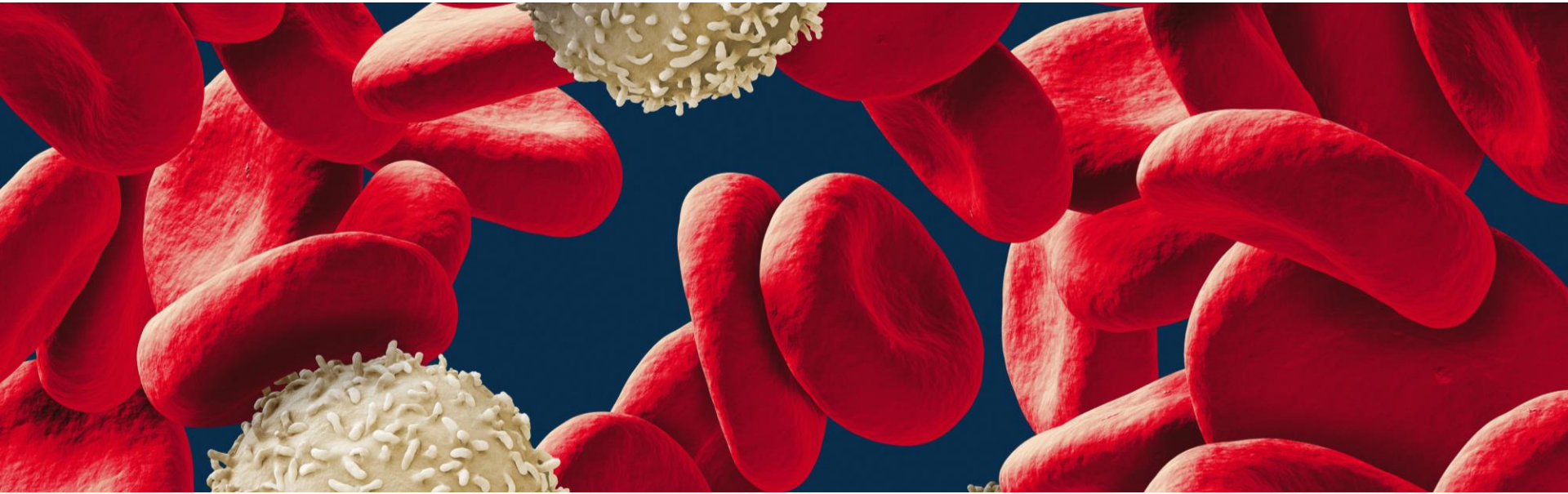
Density-gradient-free solutions for cell isolation

MACS® Cell Separation Technology

Kristina Kremer, Global Product Manager Cell Separation

August 2020



Agenda

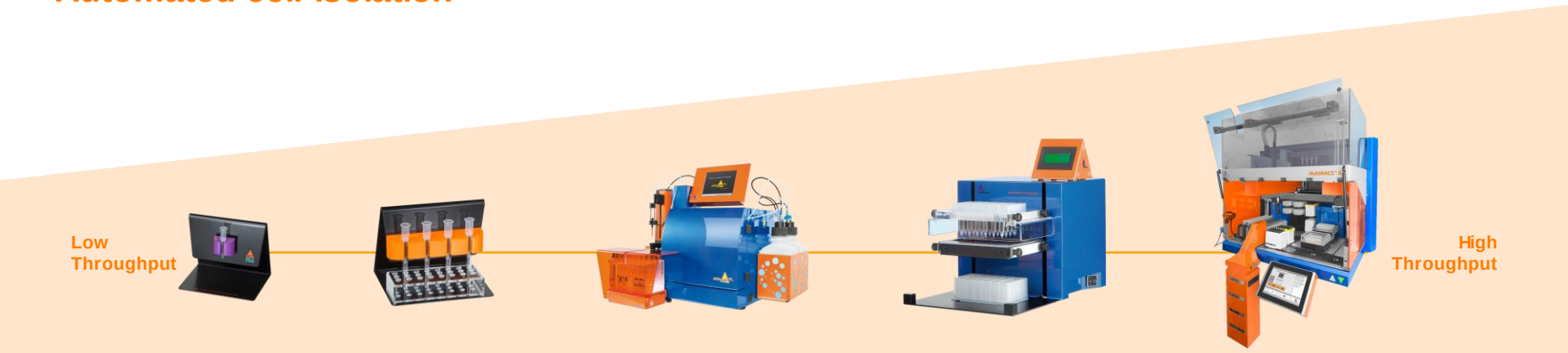
Miltenyi Biotec – pioneer in cell research and therapy

Blood products as starting material

Density-gradient centrifugation

Cell isolation of PBMCs and immune cells

Automated cell isolation



Empowering discovery
Advancing therapy

Our mission is to advance scientific research and medicine by providing solutions for curative cell and gene therapy as well as biomedical research.

Over three decades of expertise and Pioniergeist

1989

Foundation of Miltenyi Biotec.

1990

MACS® technology patent.

1994

A joint venture with Amgen is formed to develop the technology that would become the basis for the industry-changing **CliniMACS®** System.

2002

GMP manufacturing facility in Teterow opens.

2014

FDA approval **CliniMACS®** CD34 Reagent.

2020

3,000 employees worldwide.

23% of employees in global R&D.

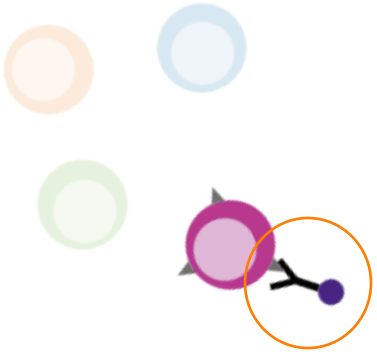
>20,000 scientific publications.

2,500 patients treated annually with cell products using Miltenyi Biotec technologies.

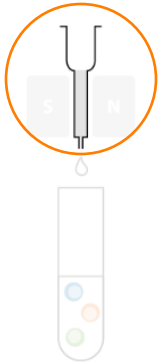
MACS® Technology:
Based on 3 components.

Miltenyi Biotec

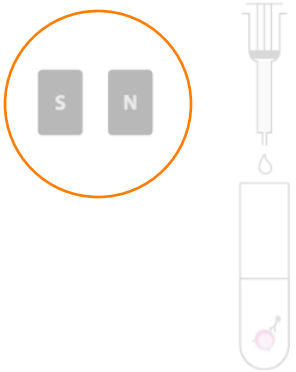
Step 1 Magnetic labeling.



Step 2 Magnetic separation.



Step 3 Elution of labeled cells.



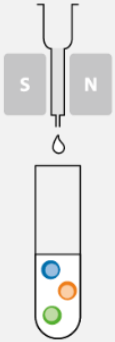
MicroBead | **Column** | **Magnetic separator**

MACS® Technology:

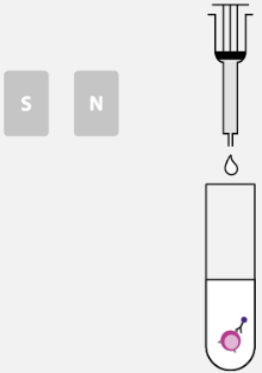
3 easy steps to viable cells for cell and gene therapy.

Step 1 Magnetic labeling.

Step 2 Magnetic separation.

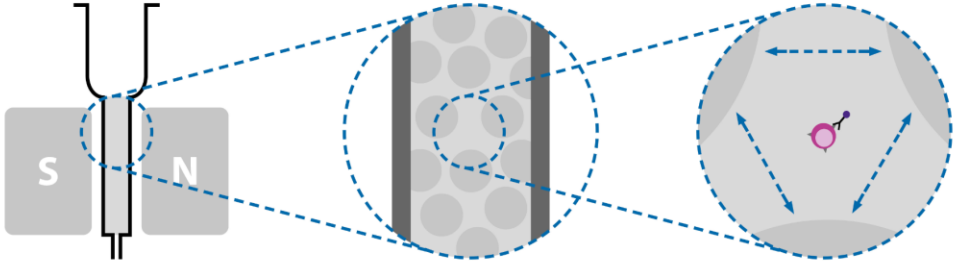


Step 3 Elution of labeled cells.



Why do we use MACS Columns?

Amplification of the gradient of the magnetic force by 10,000-fold.



Minimal labeling with nano-sized MicroBeads (50 nm).

Free flow through.

Cell-friendly hydrophilic coating.

Thorough washing for removal of cell debris and contaminants.

MACS Columns enable cell separation with minimal effect on cells for research and clinical applications.

Learn more:

Product Portfolio.
From benchtop to bedside.

Sample Preparation

Cell Isolation

Cell Activation/ Expansion

Cell Culture

Cell Analysis

Imaging

Cryo- Preservation

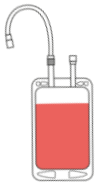
Cell Analysis

Cell Culture

Cell Activation/ Expansion

Cell Processing

Primary cells from blood products

Starting material	Sample preparation		MACS cell separation		Downstream application
					
Blood product	**Whole blood/ bone marrow**	**Buffy coat**	**Leukopak®**	**LRSC**	**Cord blood**

Blood products differ depending on the processing

Whole Blood

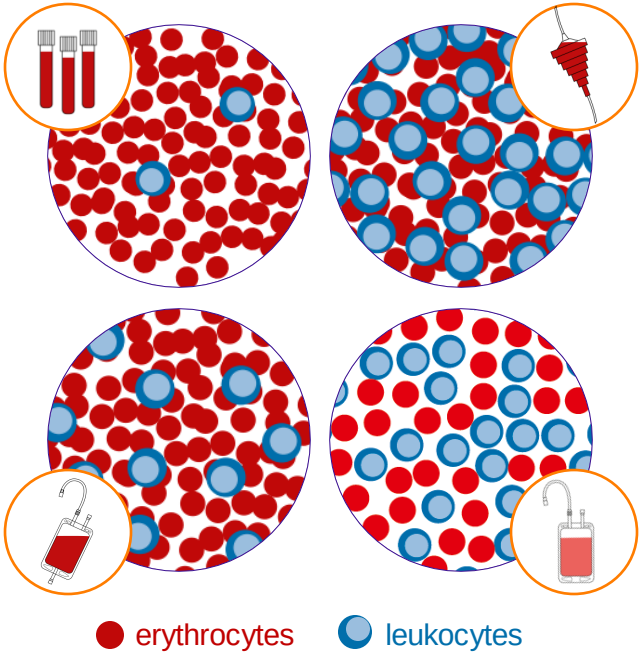
~2x10^6 PBMCs/mL
Volume: variable

Available in different volumes
Low PBMC concentration
HCT ~44%

Buffy Coat

Total PBMC ~5x10^8
Volume: 30–80 mL

Waste product after blood donation
Higher PBMC concentration
HCT ~4-10%



LRSC/Buffy cone

Total PBMC ~1x10^9
Volume: 10–15 mL

Waste product after plateletpheresis
Higher PBMC concentration
HCT ~4-10%

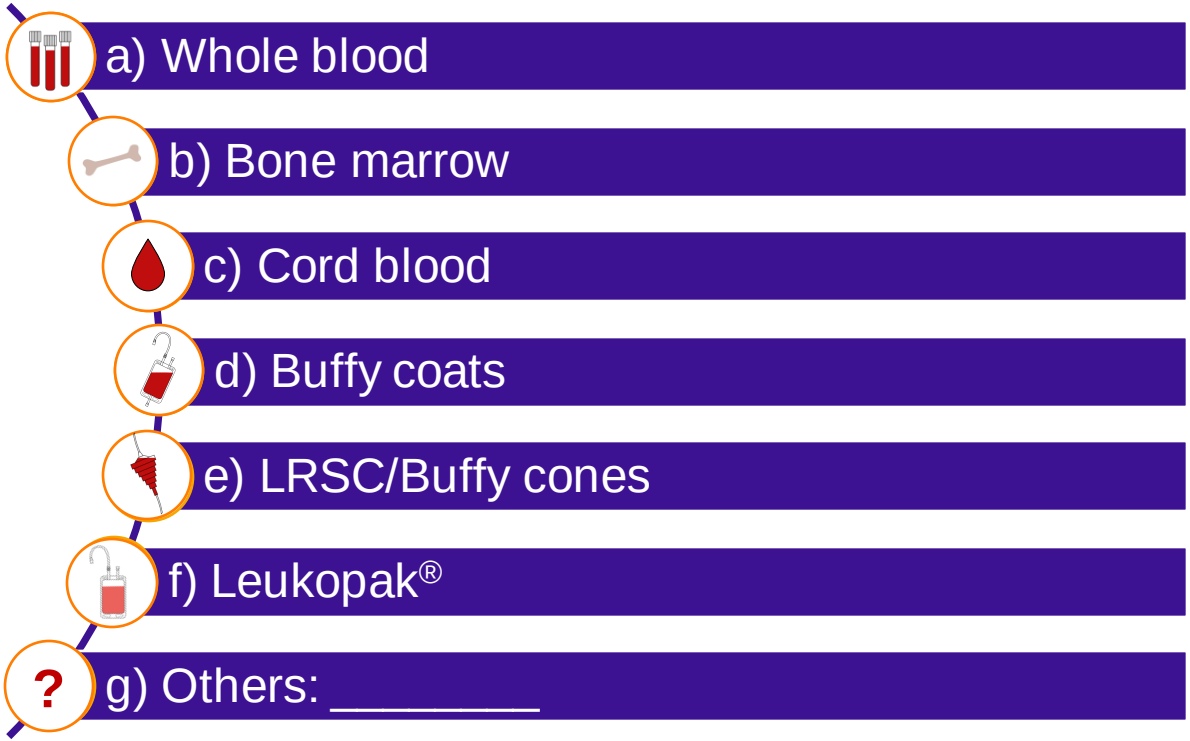
Leukopak®

Total PBMC ~1x10^{10}
Volume: 80–200 mL

Largest number of PBMCs
High PBMC concentration
HCT <3%

PBMCs = Peripheral Blood Mononuclear Cells

Q1: Which starting material do you work with primarily?



Primary cells

Starting material | **Sample preparation** | MACS cell separation | Downstream application

Blood product

- Whole blood/ bone marrow
- Buffy coat
- Leukopak®
- LRSC
- Cord blood

Density-gradient centrifugation

Primary cells

Miltenyi Biotec

Starting material | **Sample preparation** | **MACS cell separation** | **Downstream application**

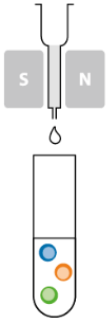
Blood product

- Whole blood/ bone marrow
- Buffy coat
- Leukopak®
- LRSC
- Cord blood

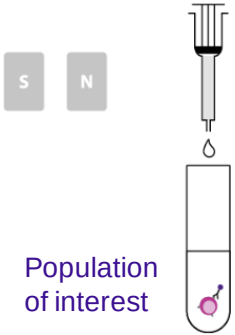
Density gradient centrifugation

1. Magnetic labeling

2. Magnetic separation



3. Elution of labeled cells



Select for downstream application:

- Positive vs. untouched isolation
- With/without Ficoll™/lysis
- Direct vs. Indirect
- Parallel vs. sequential
- Immediate vs. delayed

Primary cells

Miltenyi Biotec

Starting material

Blood product

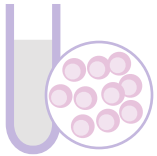
- Whole blood/ bone marrow
- Buffy coat
- Leukopak®
- LRSC
- Cord blood

Sample preparation

Density gradient centrifugation

MACS cell separation

Population of interest

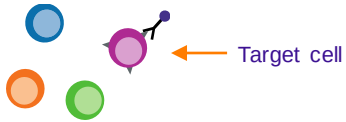


Downstream application

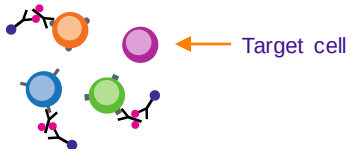
Application	Example
Additional separation	Isolation of cell subpopulations
Flow cytometry	Immuno-phenotyping
Cell culture	Functional *in vitro* assays
-omics technologies	RNA sequencing, single-cell sequencing, microarrays

Two ways of human blood cell separation

Positive isolation:
MACS® MicroBeads

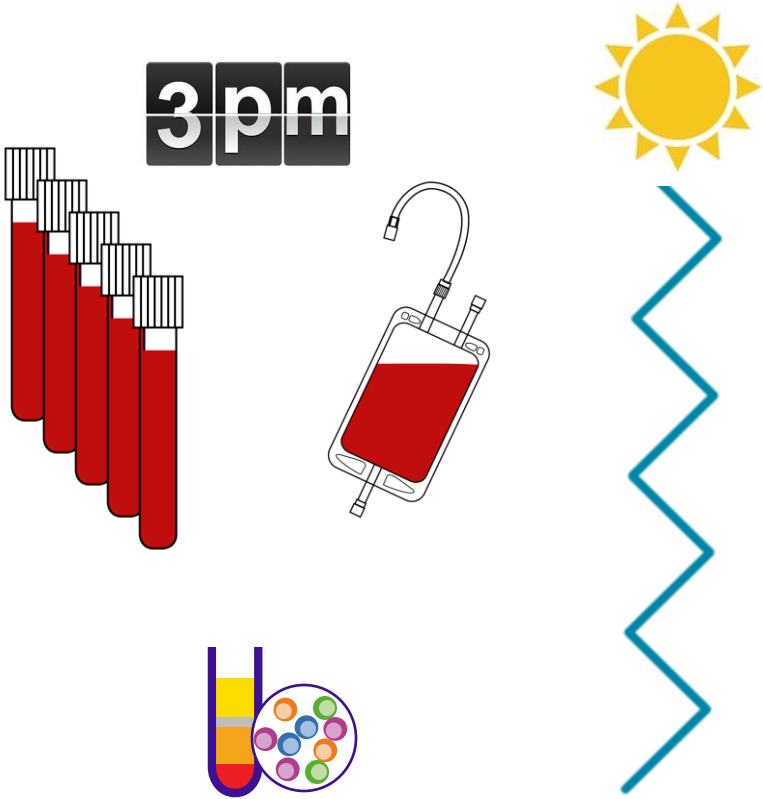


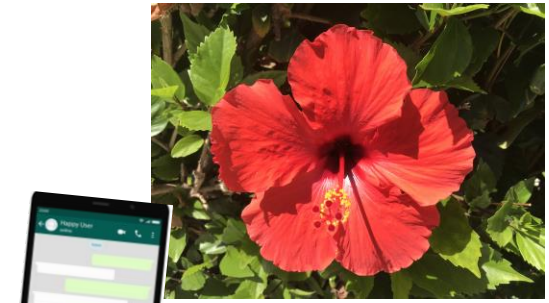
Untouched isolation:
MACS® Isolation Kits



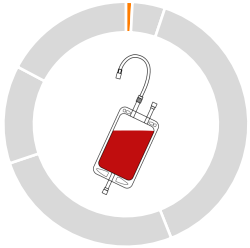
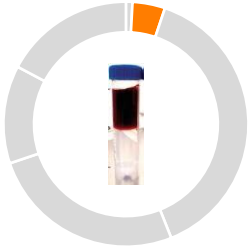
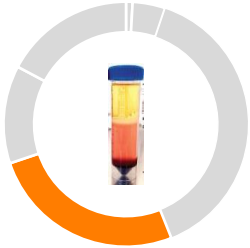
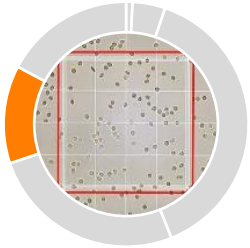
Sample to PBMC via density-gradient centrifugation

Let‘s do a quick experiment





Density-gradient centrifugation
Protocol

1 minute	5 minutes	45 minutes	30 minutes	15 minutes	20 minutes
					
Transfer and dilute sample	Layer density gradient medium	Density gradient centrifugation without brake	PBMC separation and wash	Cell count	Magnetic labeling and cell separation

2+ hours

Density-gradient centrifugation
Drawbacks

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You may have experienced:

- **RBC, platelet and/or granulocyte contamination** in separated cells or PBMCs.
- **Toxicity of Ficoll™** to White Blood Cells (WBCs).
- **Ficoll™ is cumbersome, laborious** and takes a lot of time.
- **Low recovery** of separated cells or PBMCs.
- **Intra- and interoperator variation** in the harvesting of the Ficoll™ interface.

  No automation options.

Q2: Which of the described difficulties with density gradients would you like to see solved?

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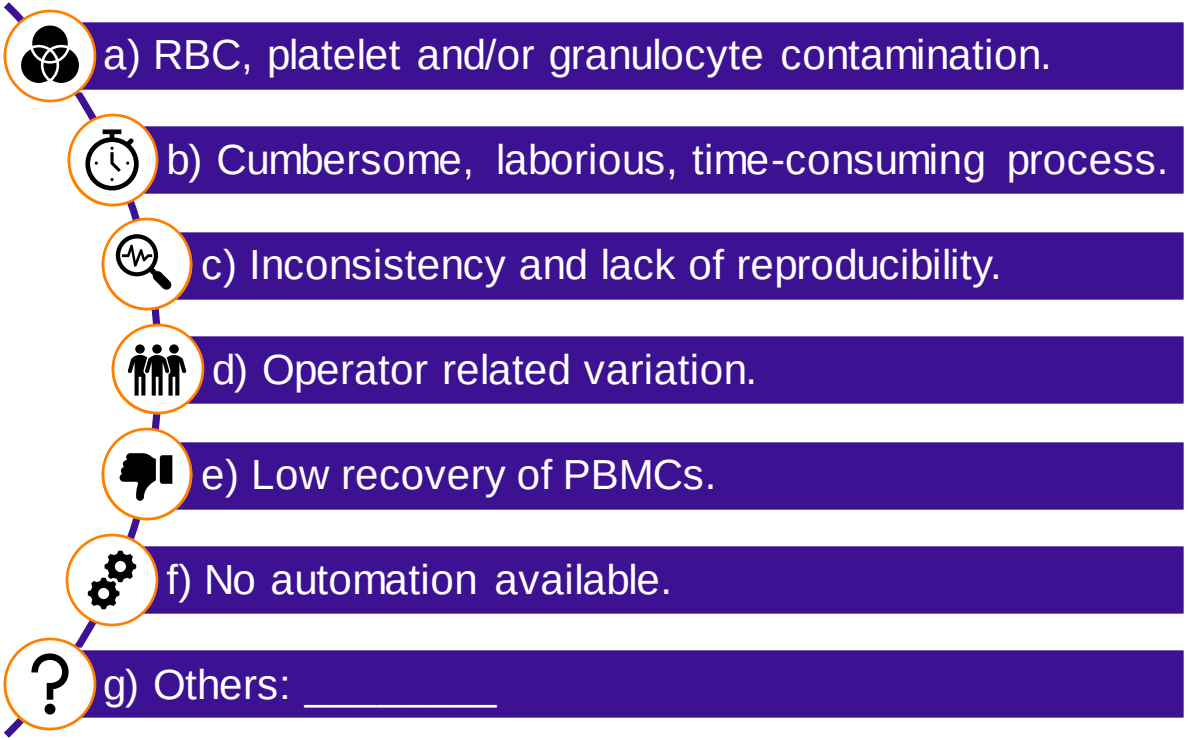

Introducing:
Cell isolations directly from your starting material

Miltenyi Biotec

Isolate PBMCs and primary cells with all the benefits:

No density-gradient centrifugation – Ficoll™-free isolations and no cell counting.

Short, easy protocols with few simple steps.

Positive and untouched isolation strategies for full flexibility.

Fastest available workflows for target cells from whole blood, buffy coat, LRSCs and Leukopaks®.

Automation available for all protocols – little hands-on, high standardization and reproducibility.

Benefits in a nutshell

Covid-19 conditions

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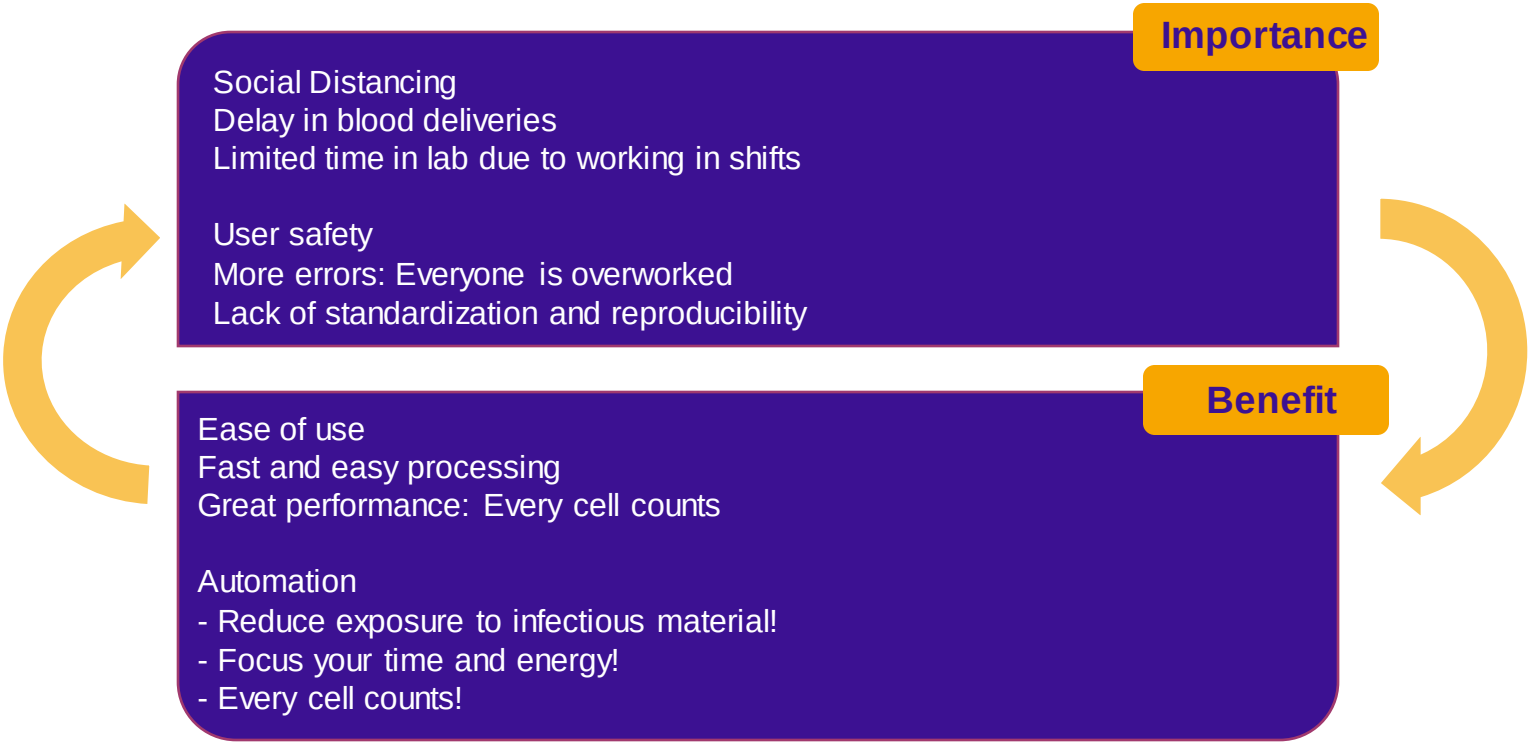
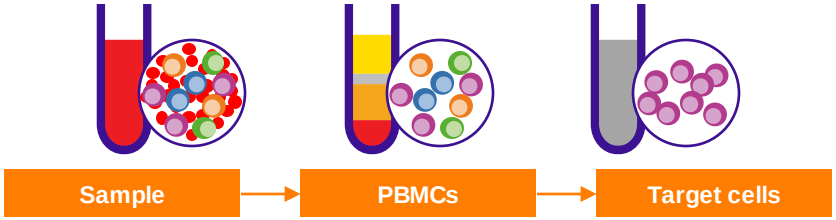
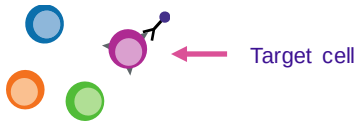


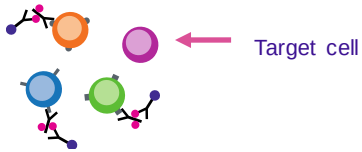
Two ways of human blood cell separation



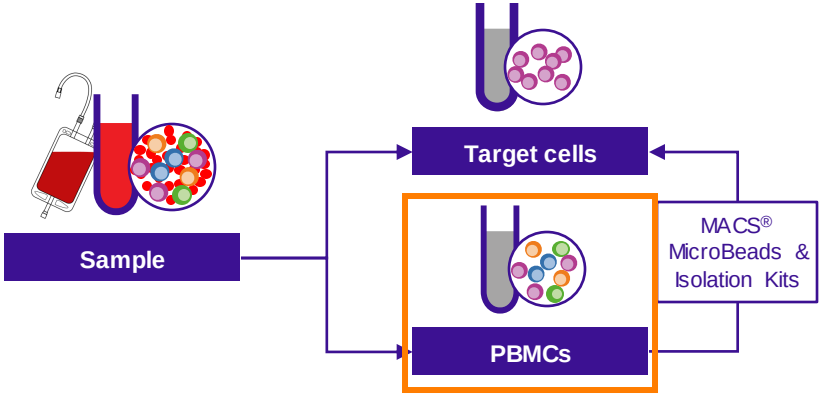
Positive isolation:
MACS® MicroBeads



Untouched isolation:
MACS® Isolation Kits



Sample to PBMC via density-gradient centrifugation



Positive or untouched isolation from

- Whole blood
- Buffy Coat
- LRSC
- Leukopak®

Directly from sample to PBMCs or target cells

Untouched PBMC isolation from blood products

Protocol

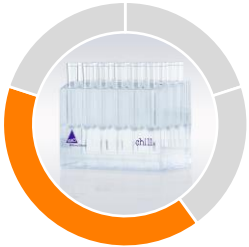
5 minutes

Sediment erythrocytes

5 minutes

Spin supernatant

10 minutes



Add reagents, incubation at 4–8°C

5-20 minutes

Separation on autoMACS® or MultiMACS™

25-40 minutes

From blood to untouched PBMCs

Untouched PBMC isolation from blood products

Time comparison

PBMC generation by density-gradient centrifugation from whole blood, buffy coat or Leukopak®

5	30	15	5	10	10

New PBMC generation method from 30mL whole blood

2	3	5	10	5

New PBMC generation method from 1 buffy coat or buffy cone

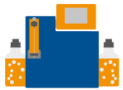
2	3	5	10	20

New PBMC generation method from 50mL Leukopak®

2	15	8

PBMC Isolation Kit

\+



autoMACS®

MultiMACS™ *

Preparation

Sedimentation

Fully automated incubation and separation

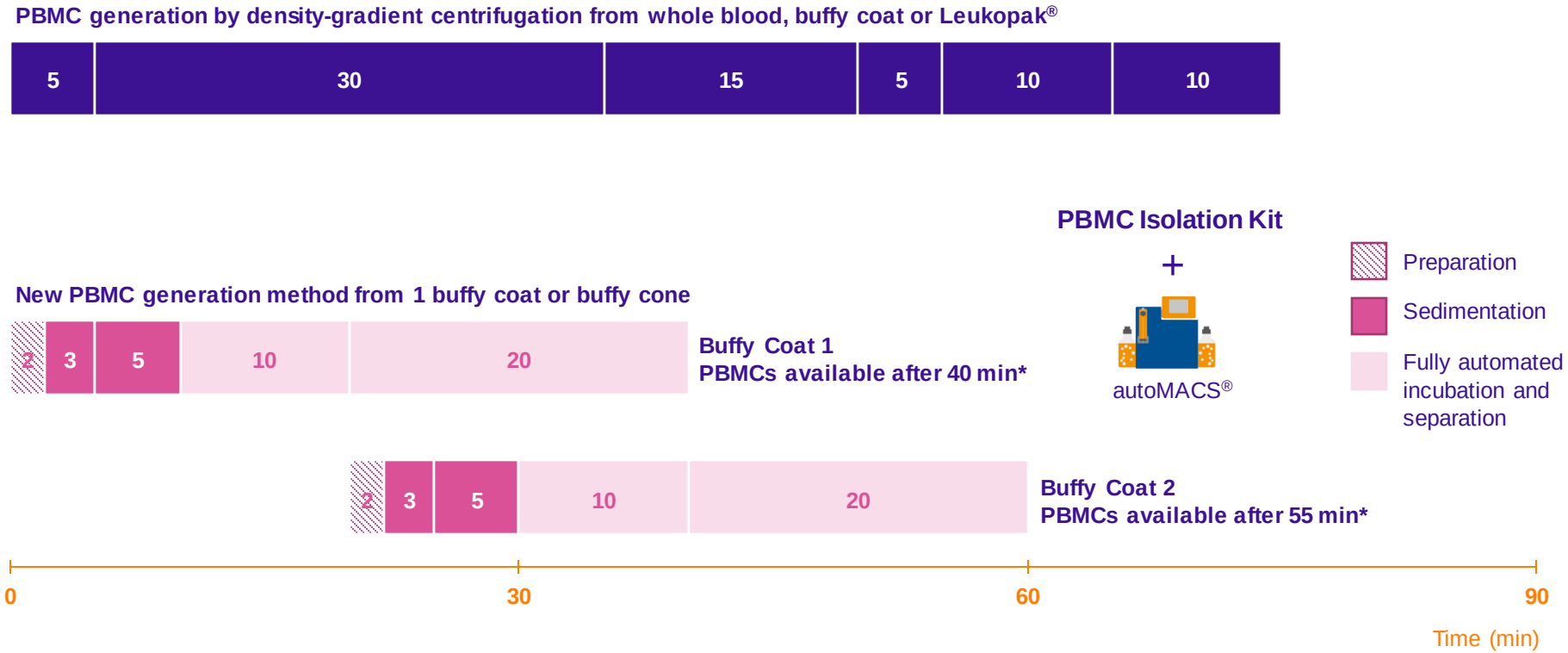
0 — 30 — 60 — 90

Time (min)

*semi-automated with MultiMACS™ Cell24 Separator Plus, fully automated with MultiMACS™ X.

Untouched PBMC isolation from buffy coats

Time comparison



*PBMCs are available after 15 min; run time includes a 5-min wash program at the end of the run

Untouched PBMC isolation from whole blood

Exemplary data

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PBMC cell type composition (in %)

Singlets		
Viable Cells	7-AAD-	
Debris Exclusion	7-AAD-	
Leukocytes	7-AAD- CD45+	
Monocytes	7-AAD- CD45+ CD14+	17.34
- Non-Classical	7-AAD- CD45+ CD14+ CD16+	4.84
- Intermediate	7-AAD- CD45+ CD14++ CD16+	6.39
- Classical	7-AAD- CD45+ CD14++ CD16-	5.12
B Cells	7-AAD- CD45+ CD14- CD19+	3.60
Eosinophils	7-AAD- CD45+ CD14- CD19- SSC hi CD16-	0.00
Neutrophils	7-AAD- CD45+ CD14- CD19- SSC hi CD16+	7.28
NK Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3- CD56+	17.99
CD3+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+	48.85
- NKT Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD56+	4.61
- CD8+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD4- CD8+	19.36
- CD4+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD4+ CD8-	27.71

Ficoll™

No variation in cell composition!

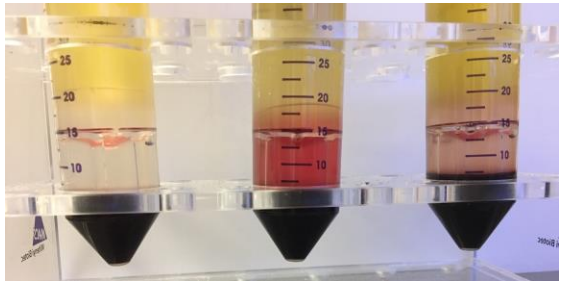
Singlets		
Viable Cells	7-AAD-	
Debris Exclusion	7-AAD-	
Leukocytes	7-AAD- CD45+	
Monocytes	7-AAD- CD45+ CD14+	16.42
- Non-Classical	7-AAD- CD45+ CD14+ CD16+	4.10
- Intermediate	7-AAD- CD45+ CD14++ CD16+	6.78
- Classical	7-AAD- CD45+ CD14++ CD16-	4.62
B Cells	7-AAD- CD45+ CD14- CD19+	3.63
Eosinophils	7-AAD- CD45+ CD14- CD19- SSC hi CD16-	0.13
Neutrophils	7-AAD- CD45+ CD14- CD19- SSC hi CD16+	0.06
NK Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3- CD56+	18.51
CD3+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+	55.83
- NKT Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD56+	6.38
- CD8+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD4- CD8+	20.89
- CD4+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD4+ CD8-	32.39

Sedimentation +

autoMACS®

Untouched PBMC isolation from whole blood

Performance comparison



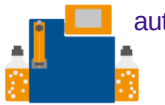
Ficoll™

	Sample 1	Sample 2	Sample 3
Granulocytes	4,90%	2,41%	1,58%
Erythrocytes	2,59%	37,81%	21,94%
Platelets	62,11%	45,34%	57,50%

Ficoll™

	Sample 1	Sample 2	Sample 3
Granulocytes	0,27%	0,29%	0,20%
Erythrocytes	0,12%	0,06%	0,06%
Platelets	66,44%	43,89%	61,01%

Sedimentation +



Untouched PBMC isolation from buffy coats

Performance comparison

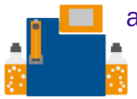
Population	Phenotype	Frequency % among leukocytes
Singlets		
Viable Cells	7-AAD-	
Debris Exclusion	7-AAD-	
Leukocytes	7-AAD- CD45+	
Monocytes	7-AAD- CD45+ CD14+	22.88
- Non-Classical	7-AAD- CD45+ CD14+ CD16+	2.10
- Intermediate	7-AAD- CD45+ CD14++ CD16+	8.19
- Classical	7-AAD- CD45+ CD14++ CD16-	11.92
B Cells	7-AAD- CD45+ CD14- CD19+	7.01
Eosinophils	7-AAD- CD45+ CD14- CD19- SSC hi CD16-	0.13
Neutrophils	7-AAD- CD45+ CD14- CD19- SSC hi CD16+	6.65
NK Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3- CD56+	5.51
CD3+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+	54.55
- NKT Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD56+	14.76
- CD8+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD4- CD8+	27.12
- CD4+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD4+ CD8-	25.46

Population	Phenotype	Frequency % among leukocytes
Singlets		
Viable Cells	7-AAD-	
Debris Exclusion	7-AAD-	
Leukocytes	7-AAD- CD45+	
Monocytes	7-AAD- CD45+ CD14+	21.96
- Non-Classical	7-AAD- CD45+ CD14+ CD16+	2.18
- Intermediate	7-AAD- CD45+ CD14++ CD16+	8.90
- Classical	7-AAD- CD45+ CD14++ CD16-	10.32
B Cells	7-AAD- CD45+ CD14- CD19+	6.00
Eosinophils	7-AAD- CD45+ CD14- CD19- SSC hi CD16-	1.13
Neutrophils	7-AAD- CD45+ CD14- CD19- SSC hi CD16+	0.01
NK Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3- CD56+	5.91
CD3+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+	61.71
- NKT Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD56+	17.44
- CD8+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD4- CD8+	32.34
- CD4+ T Cells	7-AAD- CD45+ CD14- CD19- SSC lo CD3+ CD4+ CD8-	27.05

Ficoll™

Cell type	%-T	Count/mL	Calc Tot
Viable Leukos	63.10	7.84e+05	1.57e+08
Platelets	30.26	3.76e+05	7.52e+07
RBCs	4.07	5.06e+04	1.01e+07
Grans	5.26	6.53e+04	1.31e+07

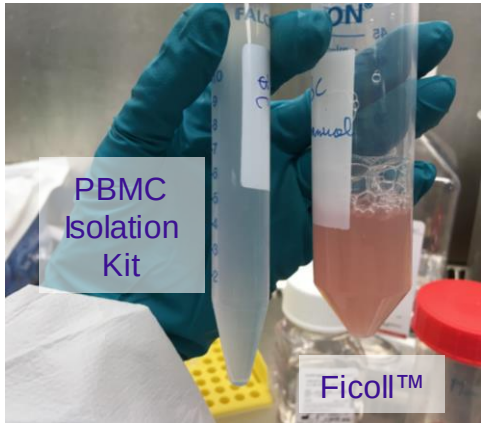
Sedimentation + autoMACS®

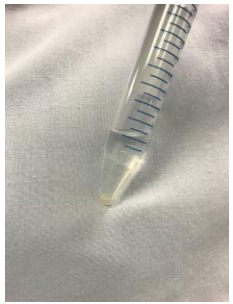


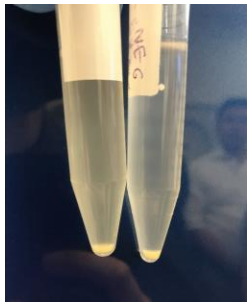
Cell type	%-T	Count/mL	Calc Tot
Viable Leukos	88.57	6.24e+05	1.25e+08
Platelets	9.15	6.45e+04	1.29e+07
RBCs	0.01	6.58e+01	1.32e+04
Grans	0.04	3.14e+02	6.28e+04

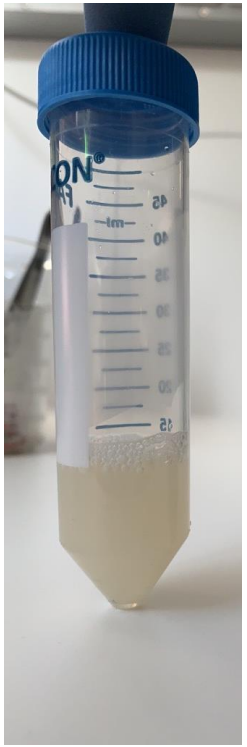
A picture is worth a thousand words

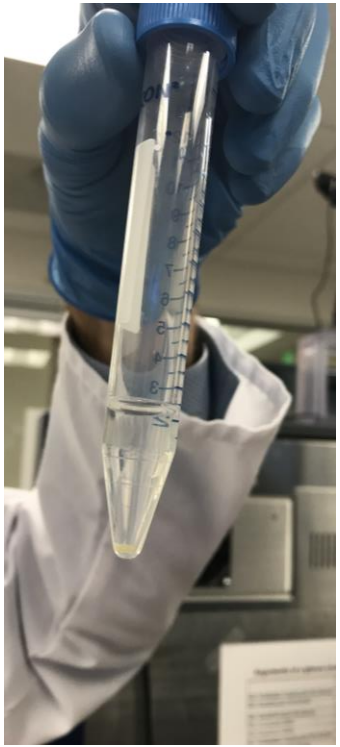
This is what our customers say



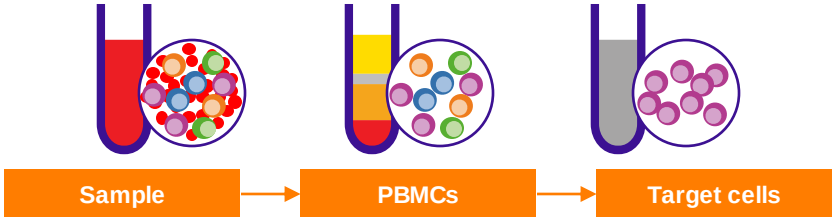




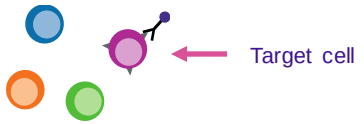




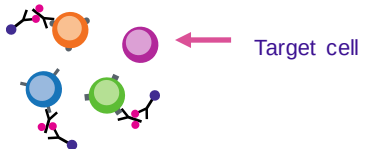
Two ways of human blood cell separation



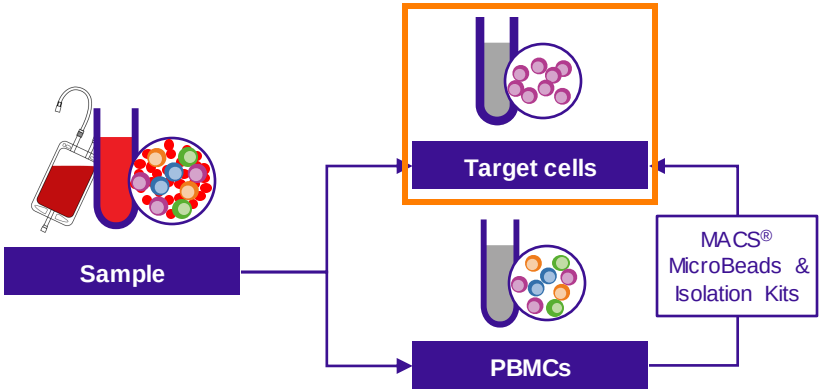
Positive isolation:
MACS® MicroBeads



Untouched isolation:
MACS® Isolation Kits



Sample to PBMC via density-gradient centrifugation



Positive or untouched isolation from

- Whole blood
- Buffy Coat
- LRSC
- Leukopak®

Directly from sample to PBMCs or target cells

StraightFrom® MicroBead Kits
Protocol

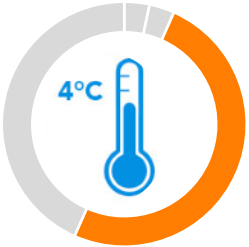
1 minute

Fill blood sample into collection tube

1 minute

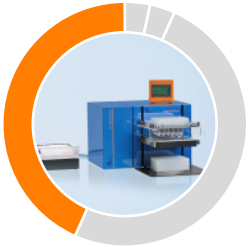
Add StraightFrom® MicroBeads

15 minutes



Incubation at 4–8°C

13 minutes



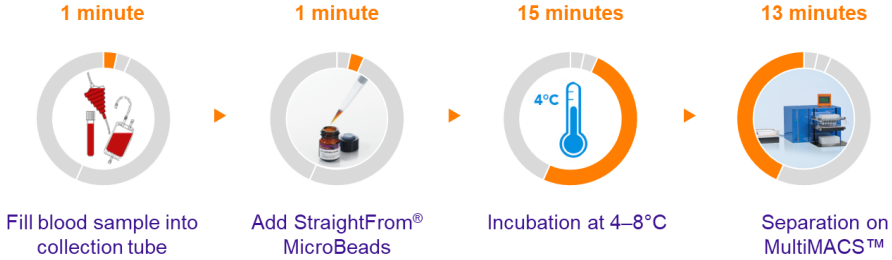
Separation on MultiMACS™ (or autoMACS®)

30 minutes

From blood product to target cells

StraightFrom® MicroBead Kits

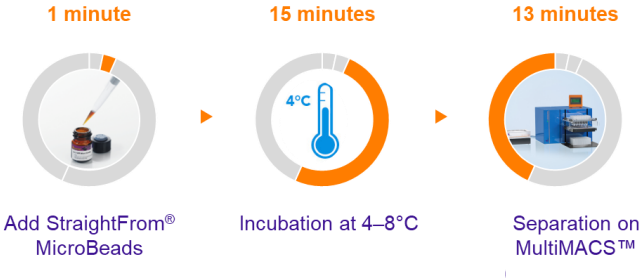
Protocol for staggering



CD8⁺ cells

StraightFrom® Leukopak® CD8 MicroBeads

Take negative fraction



CD4⁺ cells

StraightFrom® Leukopak® CD4 MicroBeads

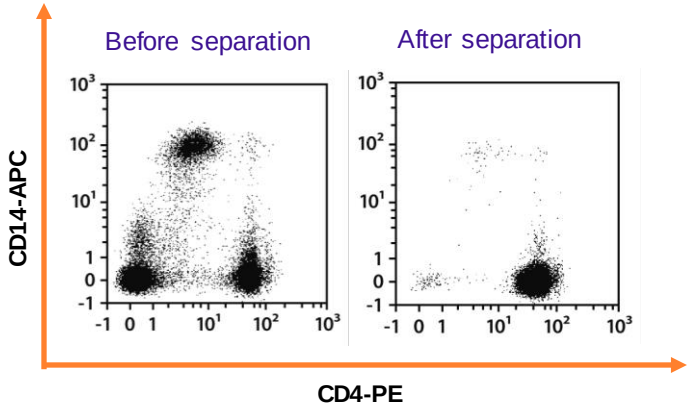
StraightFrom® MicroBead Kits
Positive isolation directly from blood products

Miltenyi Biotec

Simple and fast

- No density-gradient centrifugation
- No cell counting
- No washing after labeling

StraightFrom Leukopak® CD4 MicroBead Kit



StraightFrom® Buffy Coat MicroBeads – Exemplary data

Marker*	Purity (%)
CD3	97
CD4/CD8	98
CD4	96
CD8	95
CD14	98
CD19	95
CD56	96
…	…

*Long list of markers available on website and continuously new releases

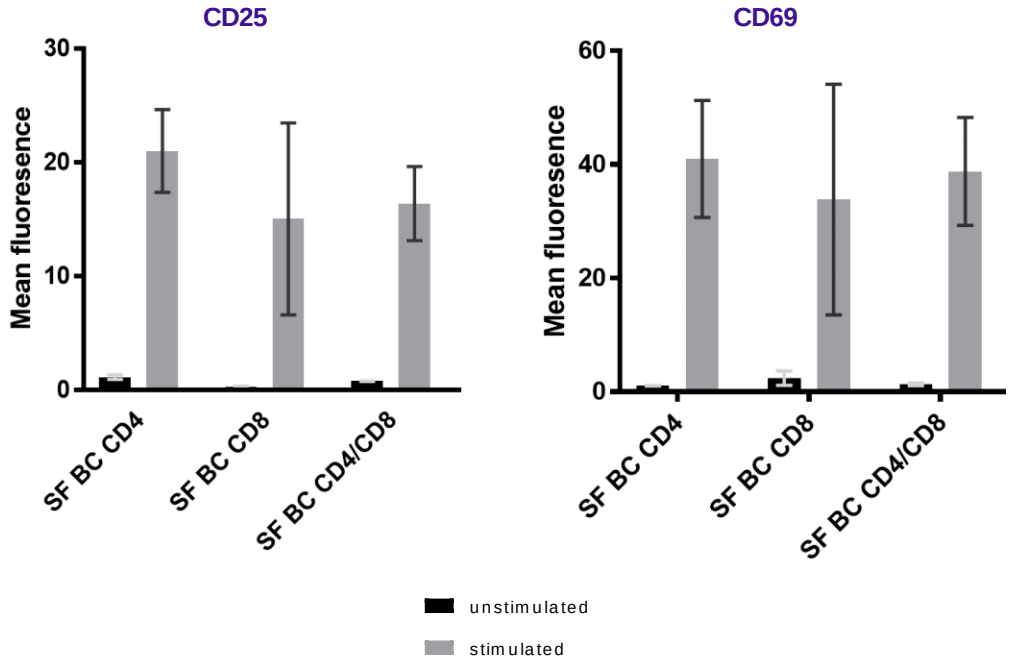
NEW

StraightFrom® Buffy Coat REAlease® MicroBead Kits, human

StraightFrom® MicroBead Kits

Performance data – activation marker analysis

StraightFrom® Buffy Coat CD4, CD8, CD4/CD8 MicroBeads

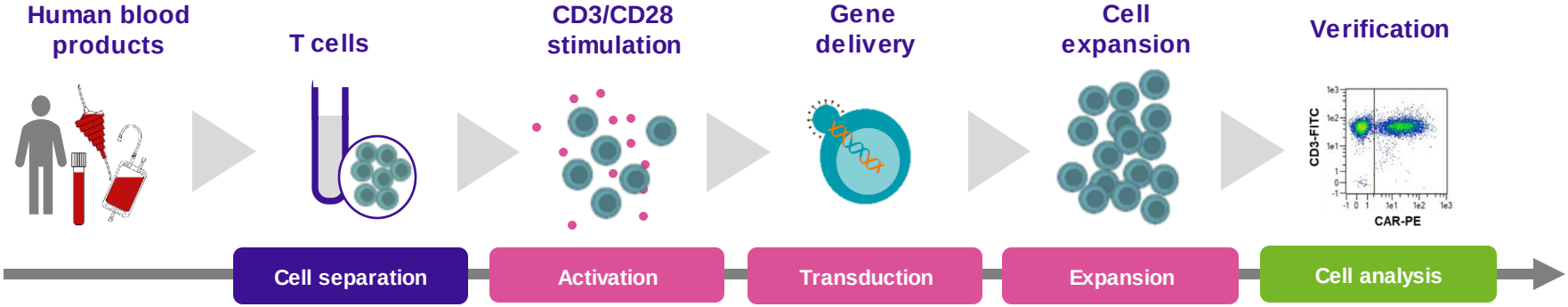


- Cells show no upregulation of activation markers
  - ➢ No activation after separation
- Successful activation upon stimulation with TransAct

Learn more:

CAR T cell engineering workflow with StraightFrom®

Application highlight



CAR T cell engineering workflow with StraightFrom®

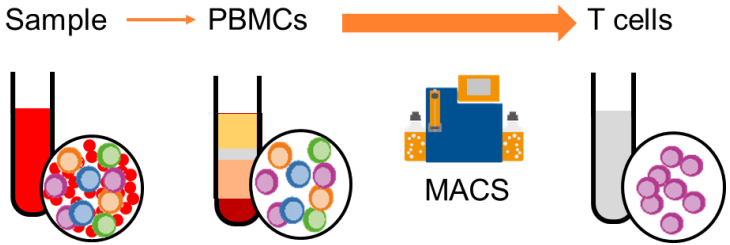
Isolation of T cells from blood products

Miltenyi Biotec

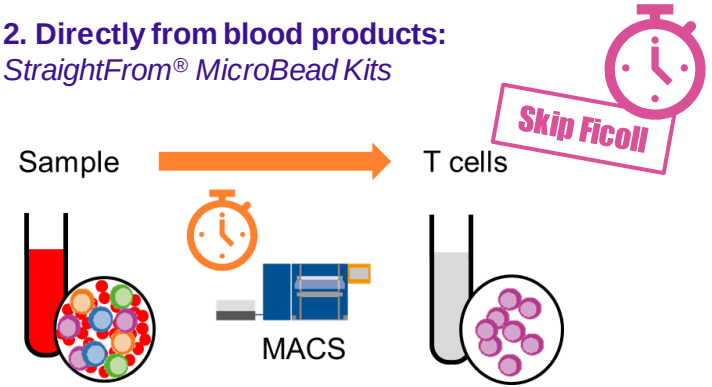
Cell separation | Activation | Transduction | Expansion | Cell analysis

MACS® MicroBeads versus StraightFrom® MicroBeads: same results, much faster

1. From PBMCs:
MACS® MicroBeads (or Isolation Kits)



2. Directly from blood products:
StraightFrom® MicroBead Kits



- ✓ *Skip density gradient centrifugation*
- ✓ *From blood to highly pure T cells within 30 min.*
- ✓ *Whole blood, buffy coat, LRSC, Leukopak®*

CAR T cell engineering workflow with StraightFrom®

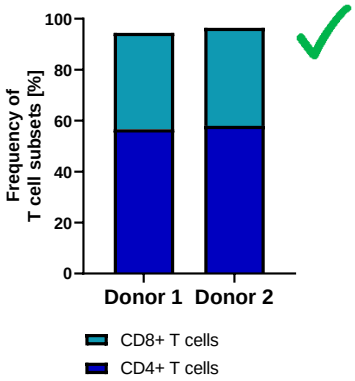
Isolation of T cells from blood products

Miltenyi Biotec

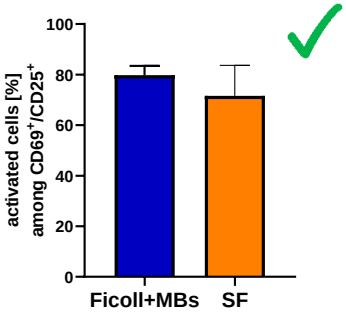
Cell separation | Activation | Transduction | Expansion | Cell analysis

MACS® MicroBeads versus StraightFrom® MicroBeads: same results, much faster

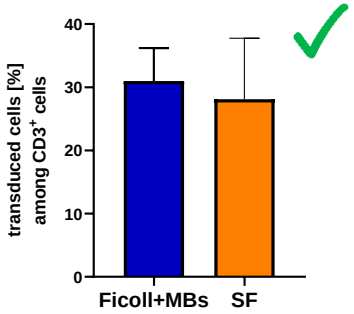
T cell enrichment



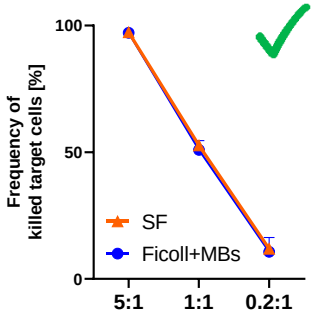
T cell activation



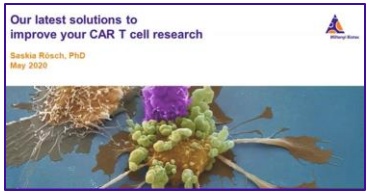
Transduction efficiency



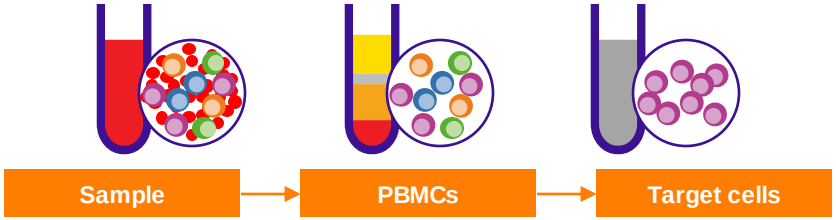
Target cell killing



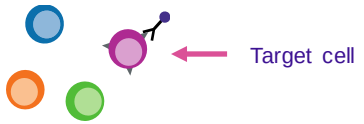
Learn more:



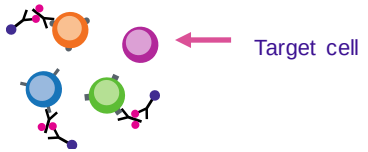
Two ways of human blood cell separation



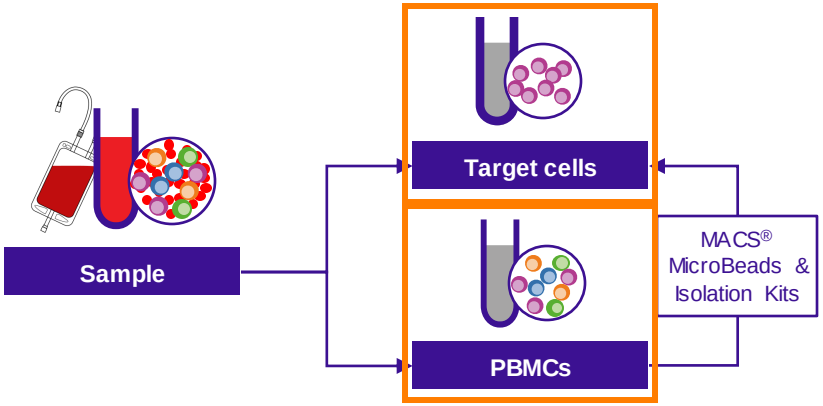
Positive isolation:
MACS® MicroBeads



Untouched isolation:
MACS® Isolation Kits



Sample to PBMC via density-gradient centrifugation



Positive or untouched isolation from

- Whole blood
- Buffy Coat
- LRSC
- Leukopak®

Directly from sample to PBMCs or target cells

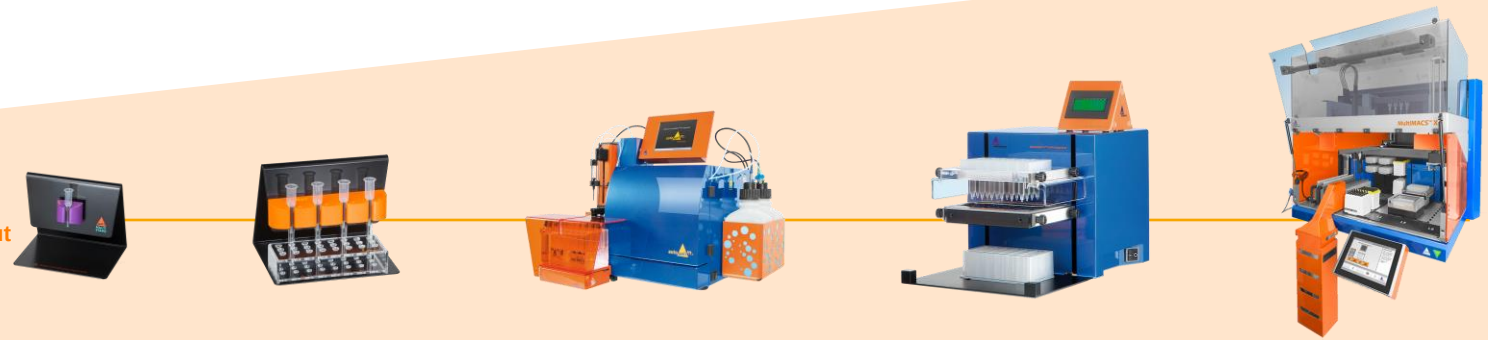
Automated high quality cell separation

Automation improves efficiency in everyday cell processing:

- **Standardization** – no user variability or human errors
- **Reproducibility** – the same protocol, the same results, same performance every time
- **Efficiency** – less hands-on time allows you to work on more important tasks
- **User safety** – reduce contaminations and employee exposure to samples

Run fewer redundant experiments,
focus your time and energy where it matters

Low Throughput



High Throughput

autoMACS® Pro Separator

Intuitive and easy-to-use automation for a multi-user lab

- Sequential separation of 1–6 samples per run
- Re-usable columns reduce costs and allow larger samples to be processed in one run
- Perfect fit for automated isolation of PBMCs from whole blood and buffy coats
- Perfect match to isolate target cells from PBMCs

MultiMACS™ Cell24 Separator Plus

Parallel cell separation of large sample numbers and high sample volumes

- Semi-automated cell separation
- Scalable sample throughput fits every workflow: 1-24 samples in one run
- Compatible with manual columns and Multi-24 Column Block
- Use the 24 magnets as individual magnets for small samples, or as one for large-volume samples such as buffy coats and Leukopaks®
- Tailored programs for isolations of cell subpopulations directly from blood products

MultiMACS™ X

Fully automated cell isolation for the demanding high-throughput cell processing laboratory

- Full automation of all relevant cell isolation workflows – PBMC and cell subpopulation isolation from Leukopaks®, Buffy Coats, Whole Blood and LRSCs
- Fully automated sample and buffer handling, magnetic labeling and separation
- Tailored programs ensure the perfect fit into your protocols, including preparation of downstream applications

Automated solutions for every blood product

Miltenyi Biotec

autoMACS® Pro Separator

1-6 samples/run

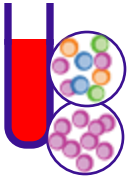
Sample volume

Level of automation

PBMC generation from whole blood & buffy coat

Isolation of cell subsets from whole blood samples

Low throughput cell subsets from PBMC aliquots



MultiMACS™ X

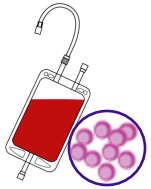
1-24 samples/run

Sample volume

Level of automation

PBMC generation from Leukopaks®

Isolation of cell subsets from buffy coat and Leukopak® samples



MultiMACS™ Cell24 Separator Plus

1-24 samples/run

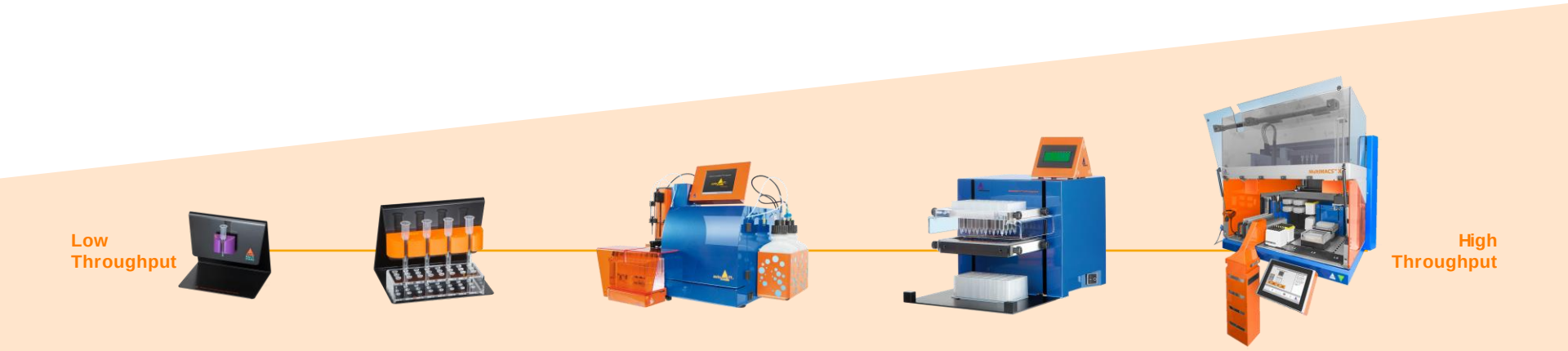
Sample volume

Level of automation

High throughput cell subsets from PBMC aliquots

Summary

- Reliable assay results depend on the right cell preparation steps and isolation strategies
- Automated solutions and Ficoll™-free protocols reduce human error and improve efficiency
- With Miltenyi Biotec‘s new density-gradient-free solutions you can isolate target cells or PBMCs directly from blood products in as little as 25 min



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Miltenyi Biotec

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Thank you!

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