Miltenyi Biotec

Clinical research Treg cell manufacture

CliniMACS® Cell Separation System and MACS® GMP Products

- Treg cell enrichment and depletion
- MACS GMP Fluorescent Antibodies for flow sorting
- Treg cell expansion
- Analysis tools for Treg cells

Clinical Treg cell research – the complete workflow

Naturally occurring $CD4^+CD25^+FoxP3^+$ regulatory T cells (Treg cells) have been shown to play an important role in[5]

- Autoimmunity
- Tolerance induction after solid organ transplantation
- Stem cell transplantation (SCT) for the prevention of graft-versus-host disease (GVHD)
- Tumor immunity

First clinical trials have been completed and Treg cells have demonstrated promising results with respect to prevention of GVHD and safety in HLA-haploidentical SCT[6] and umbilical cord blood transplantation,[9] respectively.

Contents

Quality products for clinical *ex vivo* cell processing

Miltenyi Biotec – your premium partner for cell therapy

CliniMACS® System

The CliniMACS® System offers a flexible platform for processing and separation of virtually any cell type as well as customized processing protocols. Unique reagents and tubing sets support the translation of promising research applications into innovative cell therapy concepts. Summaries of many different applications are provided on the CliniMACS System specification sheets.

The CliniMACS Prodigy® integrates MACS® Technology into a fully automated cell processing device that offers advanced solutions for streamlining cell processing workflows.

- Manufactured and controlled under an ISO 13485 quality system
- CE-marked devices in Europe
- Proven track-record of the CliniMACS Plus Cell Separation System with over 35,000 cell separations worldwide.

Figure 1: The CliniMACS Plus Instrument and the CliniMACS Prodigy.

MACS® GMP Products

MACS® GMP Products are ancillary materials/raw materials used in the manufacture of a cell therapy product. MACS GMP Products are for research use and *ex vivo* cell processing. They are tightly controlled with regard to quality and documentation to ensure safety, performance, and consistency of the final cell product. For the development of applications involving cellular therapies, procedures according to GMP guidelines are mandatory. All materials used in the manufacture of a cell product, such as antibodies, cell culture bags, media, and cytokines, must comply with GMP specifications.

- Manufactured and tested under a quality management system (ISO 13485) and are in compliance with relevant GMP guidelines
- Designed according to the recommendations of the United States Pharmacopeia, chapter <1043> on ancillary materials
- No animal-derived materials are used for manufacture of these products.
- Intended for research use and *ex vivo* cell culture processing only

Regulatory Support

Product-specific documentation

- Package Insert
- Batch-specific Certificate of Analysis (CoA) including animal component free statement
- Certificate of Compliance (CoC)/TSE (transmissible spongiform encephalopathies) certificate
- Product Information File (PIF)

USA

- Type II Master File (for cross-reference) for selected MACS GMP products

Worldwide (ex USA)

- Regulatory Support File (equivalent to Master File, for cross-reference)

Company Information and Quality System

Vendor Questionnaire available upon request.

Expert consultation

Miltenyi Biotec actively interacts with regulatory agencies and notified bodies. Please ask us about customized support for your clinical study.

Clinical grade Treg cell enrichment and depletion

CliniMACS® CD25 Product Line

With the CliniMACS® CD25 Product Line Miltenyi Biotec provides solutions for either the depletion or enrichment of CD25 positive cells.

Processing Treg cells

Treg cells for clinical applications can be prepared using the CliniMACS Cell Separation System by enriching CD25 highly expressing cells with or without prior depletion of CD8 or CD19 positive cells.

- Robust large-scale Treg cell enrichment
- CE-marked cell separation components
- ISO 13485-certified manufacturing

References

- Treg cell enrichment for HLA-haploidentical stem cell transplantation as a means to prevent and treat GVHD,[1–8,10]
- Treg cell expansion for safety studies in adults transplanted with umbilical cord blood,[9]
- Treg cell depletion to improve graft versus tumor effect (GVL)[6] and to augment tumor immunity.[11,12]
- Treg cell enrichment followed by expansion for prevention of graft rejection in solid organ transplantation[13]

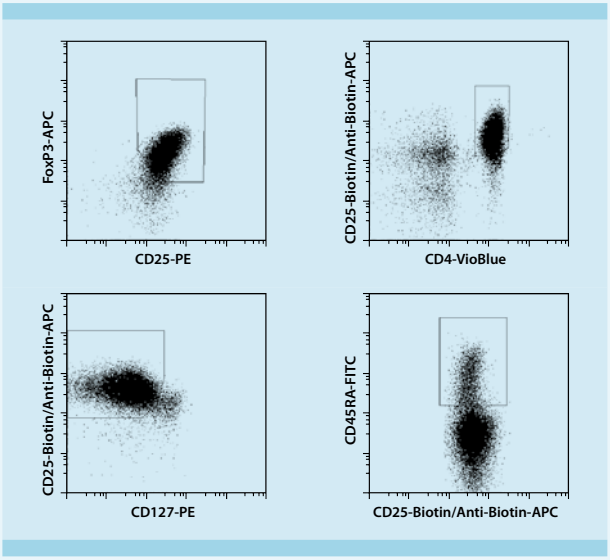
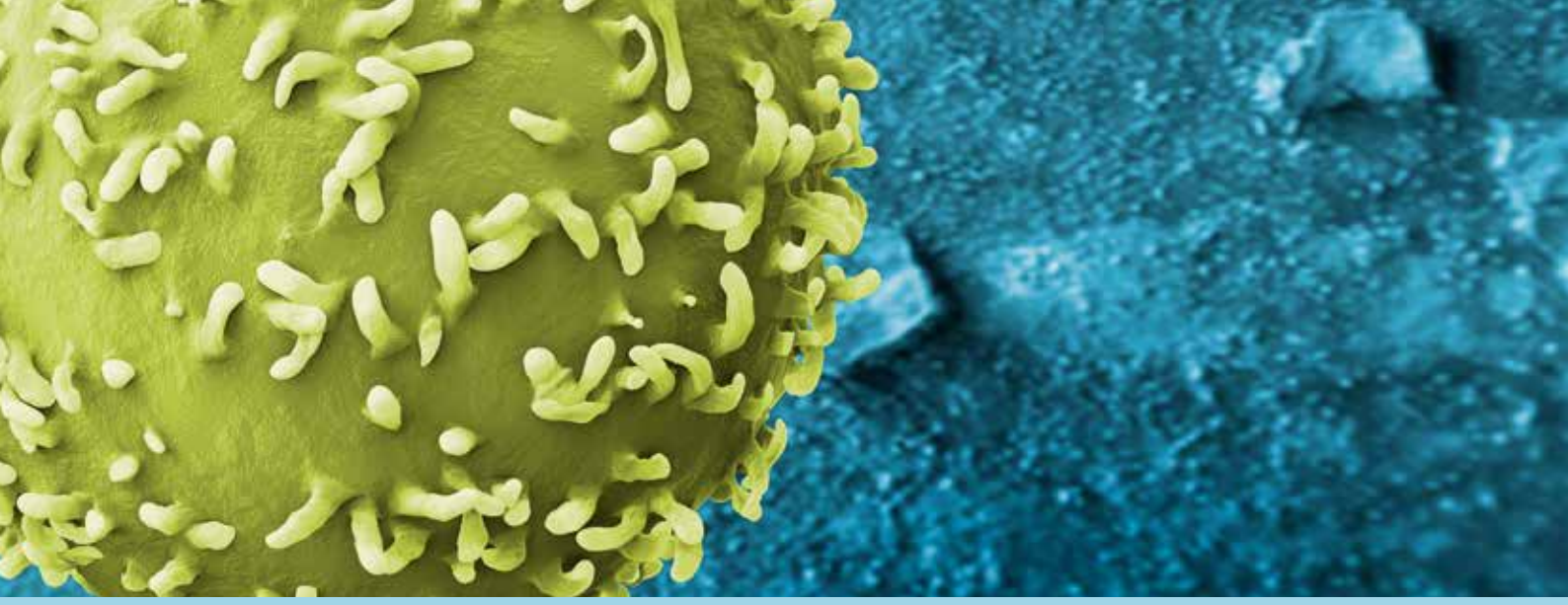


Figure 2: Clinical-scale CD25⁺ cell separation from leukapheresis products using the enrichment protocol for CD25 highly expressing cells. One representative cell sample after separation was stained for CD4 vs. CD25, CD25 vs. FoxP3 (gated on CD4⁺ T cells), CD127 vs. CD25 (gated on CD4⁺ T cells), and CD25 vs. CD45RA (gated on CD4⁺CD127⁻ T cells).

	Capacity	Total cells
Enrichment		
CD25 highly expressing cells*	0.6×10^9	40×10^9
CD25 positive cells*	0.6×10^9	40×10^9
Depletion		
CD25 positive cells	(1 vial) 6×10^9 (2 vials) 12×10^9	40×10^9 80×10^9
CD8 positive cells	(1 vial) 4×10^9	40×10^9
CD19 positive cells	(1 vial) 5×10^9	40×10^9

*populations are distinguished by labeling procedure

Table 1: Specification and capacity for the enrichment or depletion of CD25 expressing cells using the CliniMACS Cell Separation System and the CliniMACS Plus Instrument.



Flow sorting for highly purified Treg cells

MACS® GMP Fluorescent Antibodies

Enrichment of highly purified Treg cells

Precisely defined cell populations and multiparameter sorting are becoming increasingly important in the field of clinical Treg cell research.

MACS® GMP Fluorescent Antibodies have been developed for the flow cytometric detection and subsequent flow cytometric sorting of highly pure Treg cell populations in the clinical setting.

- Functionality test on every batch
- One vial per dose
- Liquid formulation
- Customized products by request

MACS GMP CD25-PE-Biotin and CliniMACS® Anti-Biotin Reagent can be used for magnetic pre-enrichment of Treg cells from large volumes, followed by flow cytometric sorting for a defined and pure final product.

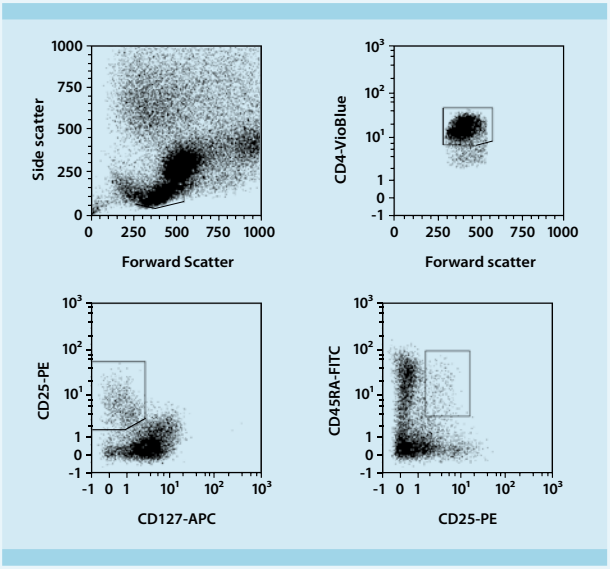


Figure 3: Staining of Treg cells with MACS GMP Fluorescent Antibodies

Product	Format	Capacity
MACS GMP CD4-VioBlue (clone M-T466)	5 mL	Up to 1×10^9 total cells
MACS GMP CD25-PE (clone 3G10)	5 mL	Up to 1×10^9 total cells
MACS GMP CD127-APC (clone MB15-18C9)	5 mL	Up to 1×10^9 total cells
MACS GMP CD45RA-FITC (clone T6D11)	5 mL	Up to 1×10^9 total cells
MACS GMP CD25-PE-Biotin* (clone 3G10)	5 mL	Up to 1×10^{10} total cells

Table 2: MACS GMP Fluorescent Antibodies for flow sorting of Treg cells

MACS GMP Products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications.

*In the US, product is available for research use only.

Efficient Treg cell expansion

GMP workflow solutions

MACS® GMP ExpAct™ Treg Kit

The MACS GMP ExpAct™ Treg Kit has been designed to efficiently expand Treg cells after a CliniMACS CD25 enrichment procedure and to maintain FoxP3 expression. It can be used with MACS GMP Rapamycin, which inhibits the expansion of conventional T cells and thereby enhances the proportion of expanded Treg cells in culture.[13–15]

- Convenient format with 1×10^9 ExpAct Treg Beads per vial sufficient for 2.5×10^8 Treg cells
- Removal of ExpAct Treg Beads with the CliniMACS® Plus Instrument and the CliniMACS Tubing Set LS

TexMACS™ GMP Medium

TexMACS™ GMP Medium is free of serum and xeno-components, and has been specifically developed for T cells and Treg cells.

- Contains pharmaceutical grade human serum albumin
- Optimized formulation without phenol red; contains glucose and stable glutamine (L-alanyl-L-glutamine)
- QC functionality test on every batch
- Filled in flexible bags (connection via Luer fitting) (see figure 4)
- Customized formats on request

Product	Format	Configuration
MACS GMP ExpAct Treg Kit	2 vials	1 vial contains 1×10^9 ExpAct Treg Beads in 5 mL
TexMACS GMP Medium	Flexible bag	2000 mL
MACS GMP Rapamycin (200 nM)	1 vial	1 mL
MACS GMP Recombinant Human IL-2	1 vial 1 vial	20 µg 100 µg
MACS GMP Expansion Bag	5 bags	up to 100 mL
MACS GMP Cell Differentiation Bag -100 -250 -500 -1000 -3000	 5 bags 5 bags 5 bags 5 bags 5 bags	 100 mL 200 mL 500 mL 1000 mL 3000 mL

Table 3: MACS GMP Products for the expansion of Treg cells.

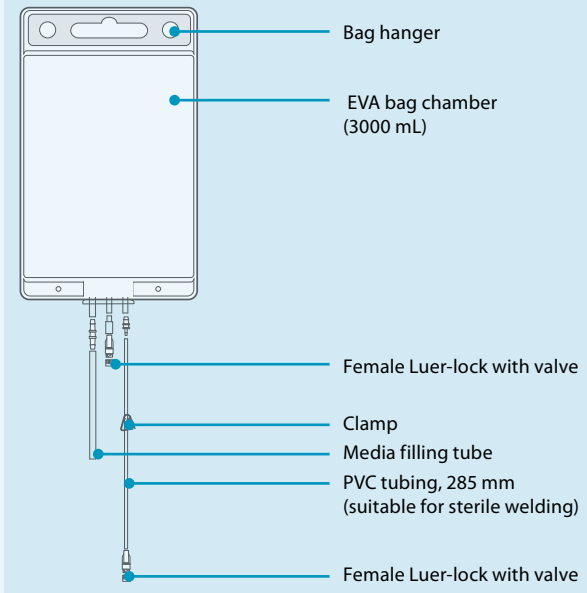


Figure 4: MACS GMP media bag.

Tools for cell analysis

Treg cell detection and analysis

Treg Detection Kits (RUO*)

The Treg Detection Kit (CD4/CD25/CD127) is a simple one-reagent tool for the identification of Treg cells before and after CliniMACS® CD25 enrichment or depletion.

It includes monoclonal antibodies against the standard Treg cell markers CD4, CD25, and CD127 as well as the leukocyte marker CD45 at optimized titers, all in one vial.

The combination of CD4, CD25, and CD127 staining is the current standard method for the identification of live Treg cells. Several publications have reported a strong correlation between the $CD25^+CD127^{dim/-}$ phenotype, expression of FoxP3, and suppressive function of $CD4^+$ cells.[16,17]

- Convenient one vial format
- CD25 clone (4E3) recognizes a different epitope from that recognized by the CliniMACS CD25 Reagent

Product	Configuration
Treg Detection Kit (CD4/CD25/CD127)	25 tests 100 tests
Treg Detection Kit (CD4/CD25/FoxP3) (PE)	100 tests
Treg Detection Kit (CD4/CD25/FoxP3) (APC)	100 tests
Treg Suppression Inspector	2.5 mL, 5×10^7 MACSiBead Particles

Table 4: Products for the analysis of Treg cells.

Treg Suppression Inspector (RUO*)

The ideal solution for the functional analysis of human Treg cells, the Treg Suppression Inspector stimulates the proliferation of responder T cells ($CD4^+CD25^-$ or $CD4^+$ T cells or PBMCs). Co-culture of Treg cells with T responder cells in the presence of the Treg Suppression Inspector results in reduced proliferation of the T responder cells due to the suppressive function of the Treg cells.

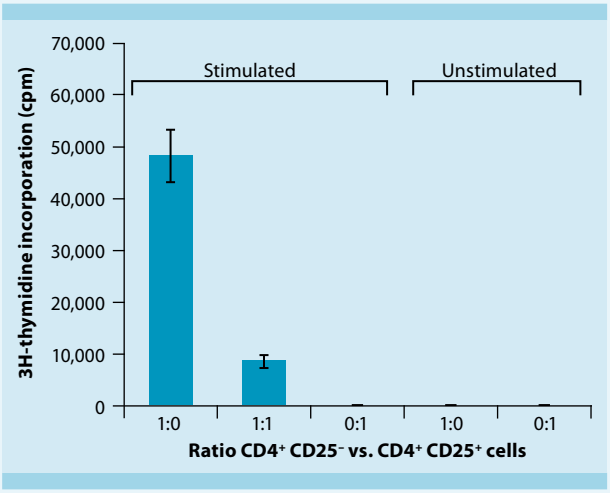
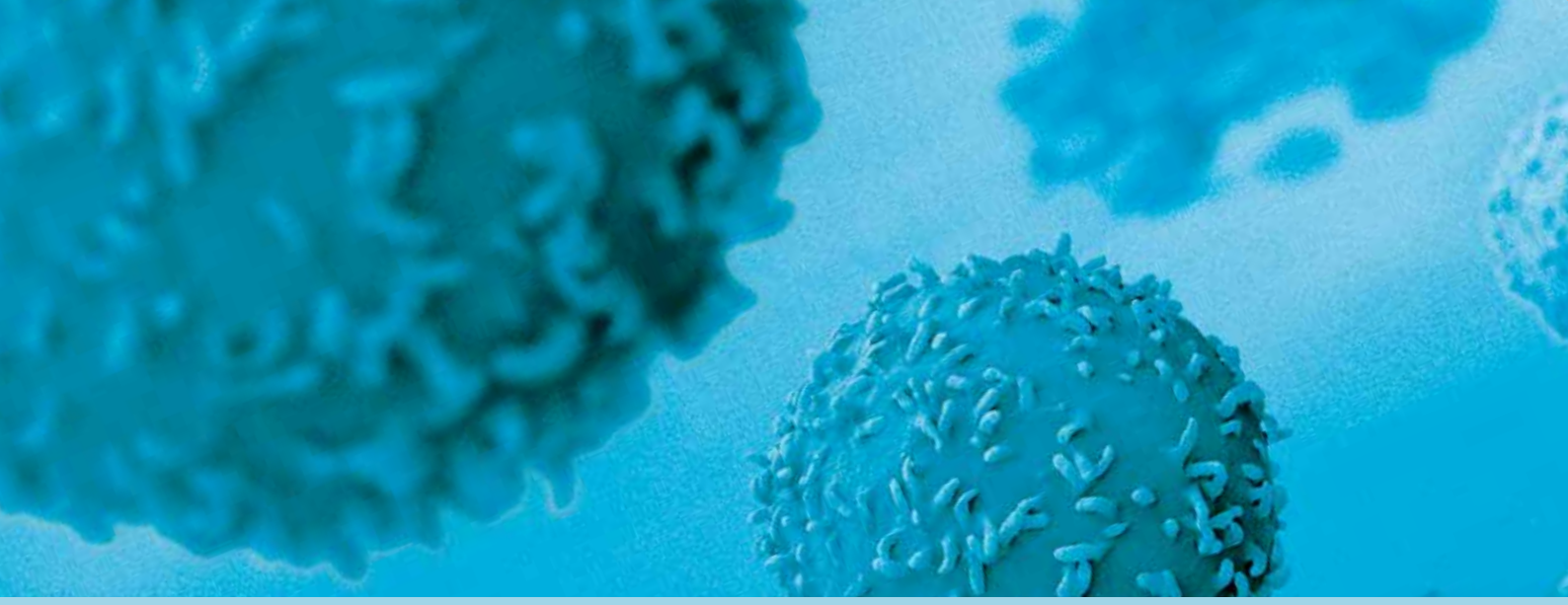


Figure 5: Functional analysis of Treg cells using the Treg Suppression Inspector. Human Treg cells, isolated with the $CD4^+CD25^+$ Regulatory T Cell Isolation Kit, were co-cultured with $CD4^+CD25^-$ responder T cells at different ratios. For T cell stimulation, the Treg Suppression Inspector was added to the culture. As controls, Treg and responder T cells alone were cultured without any stimulus. Proliferation of T cells was determined by measuring ^{3}H-thymidine incorporation.

* RUO = research use only



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2. Martelli M.F. *et al.* (2014) HLA-haploidentical transplantation with regulatory and conventional T-cell adoptive immunotherapy prevents acute leukemia relapse. Blood 124: 638–644.
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4. Theil A. *et al.* (2015) Adoptive transfer of allogeneic regulatory T cells into patients with chronic graft-versus-host disease. Cytother. 17: 473–486
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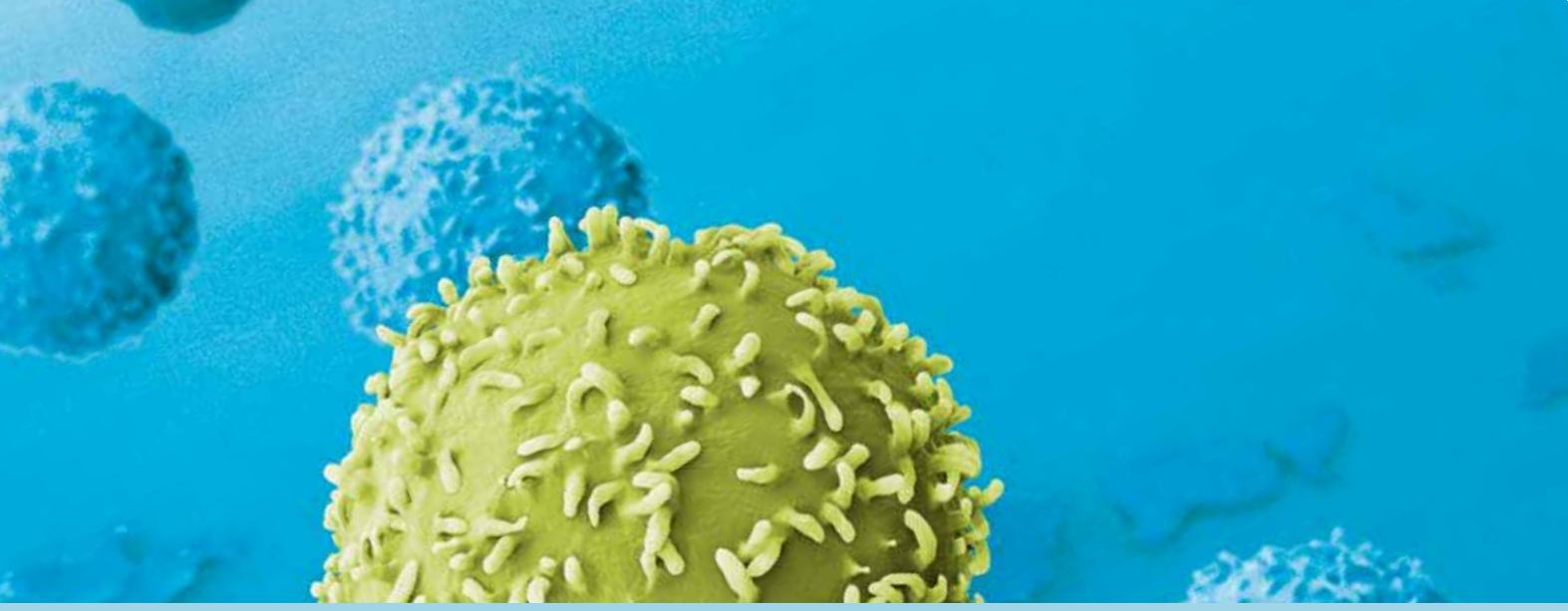
10. Maury, S. *et al.* (2010) $CD4^+CD25^+$ regulatory T cell depletion improves the graft-versus-tumor effect of donor lymphocytes after allogeneic hematopoietic stem cell transplantation. Sci. Transl. Med. 2: 41ra52.
11. Thistlethwaite, F.C. *et al.* (2008) Adoptive transfer of T(reg) depleted autologous T cells in advanced renal cell carcinoma. Cancer Immunol. Immunother. 57: 623–634.
12. Powell, D.J. Jr. *et al.* (2007) Inability to mediate prolonged reduction of regulatory T Cells after transfer of autologous CD25-depleted PBMC and interleukin-2 after lymphodepleting chemotherapy. J. Immunother. 30: 438–447.
13. Fazekasova H. *et al.* (2013) Clinical-grade separation and expansion of regulatory T cells for clinical studies on cell therapy in solid organ transplantation. Excerpt from MACS&more; Vol 15–2.
14. Putnam A.L. *et al.* (2013) Clinical grade manufacturing of human alloantigen-reactive regulatory T cells for use in transplantation. Am. J. Transplant. 13: 3010–3020.
15. Battaglia, M. *et al.* (2005) Rapamycin selectively expands $CD4^+CD25^+FoxP3^+$ regulatory T cells. Blood 105: 4743–4748.
16. Seddiki, N. *et al.* (2006) Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J. Exp. Med. 203: 1693–1700.
17. Liu, W. *et al.* (2006) CD127 expression inversely correlates with FoxP3 and suppressive function of human $CD4^+$ T reg cells. J. Exp. Med. 203: 1701–1711.

For the complete reference list, please refer to **www.clinimacs.com**



Complete workflow for clinical Treg cell research

Performance, consistency, and compliance




Leukapheresis	Cell separation	Cell expansion or differentiation	Cell analysis	Cryopreservation	Ready to use cellular product
	CliniMACS® Instruments	MACS GMP Media	MACSQuant Instruments (RUO)	CryoMACS® Freezing Bags	
	CliniMACS Reagents	MACS GMP Activation Kits	MACS Antibodies (RUO)	CryoMACS DMSO 10 (EP, USP)	
	MACSQuant® Tyto™ (RUO)	MACS GMP Expansion Tools			
	MACS® GMP Fluorescent Antibodies	MACS GMP Antigens			
		MACS GMP Cytokines			
		MACS GMP Cell Culture Bags			

RUO = Research Use Only
EP = European Pharmacopoeia
USP = United States Pharmacopeia

MACS GMP Products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications. For regulatory status in the US, please contact your local representative. MACS GMP Products are manufactured and tested under a quality management system (ISO 13485) and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials.

Ordering information

CliniMACS® Plus Cell Separation System

Product	Components	Regulatory status	Availability	Order no.
CliniMACS Anti-Biotin Reagent	7.5 mL	CE	Europe, USA[1], other[2]	173-01
CliniMACS CD8 Reagent	7.5 mL	CE	Europe, USA[1], other[2]	275-01
CliniMACS CD8 MicroBeads	7.5 mL	For research use only, not for use in humans	Worldwide[2]	308-01
CliniMACS CD19 Reagent	7.5 mL	CE	Europe, USA[1], other[2]	179-01
CliniMACS CD19 MicroBeads	7.5 mL	For research use only, not for use in humans	Worldwide[2]	193-01
CliniMACS CD25 Reagent	7.5 mL	CE	Europe, USA[1], other[2]	274-01
CliniMACS CD25 MicroBeads	7.5 mL	For research use only, not for use in humans	Worldwide[2]	325-01
CliniMACS Tubing Set TS	1 Tubing Set	CE	Europe, CAN, AUS, USA[1], Other[2]	161-01
CliniMACS Tubing Set For Research Use	1 Tubing Set	For research use only, not for use in humans	Worldwide[2]	165-01
CliniMACS Tubing Set LS	1 Tubing Set	CE	Europe, CAN, AUS, USA[1], Other[2]	162-01
CliniMACS Tubing Set LS For Research Use	1 Tubing Set	For research use only, not for use in humans	Worldwide[2]	168-01
CliniMACS PBS/EDTA Buffer	3×1 L plastic bag	CE	Europe, CAN, AUS, USA[1], other[2]	700-25
CliniMACS PBS/EDTA Buffer For Research use	3×1 L plastic bag	For research use only, not for use in humans	Worldwide[2]	705-25

[1] In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Set TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved; all other products of the CliniMACS product line are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE).
CliniMACS MicroBeads are for research use only and not for human therapeutic or diagnostic use. Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research only and not for therapeutic or diagnostic use.
[2] For availability in your country please contact your local representative.

For other CliniMACS Acessories or Products please visit **www.clinimacs.com**

MACS® GMP Products

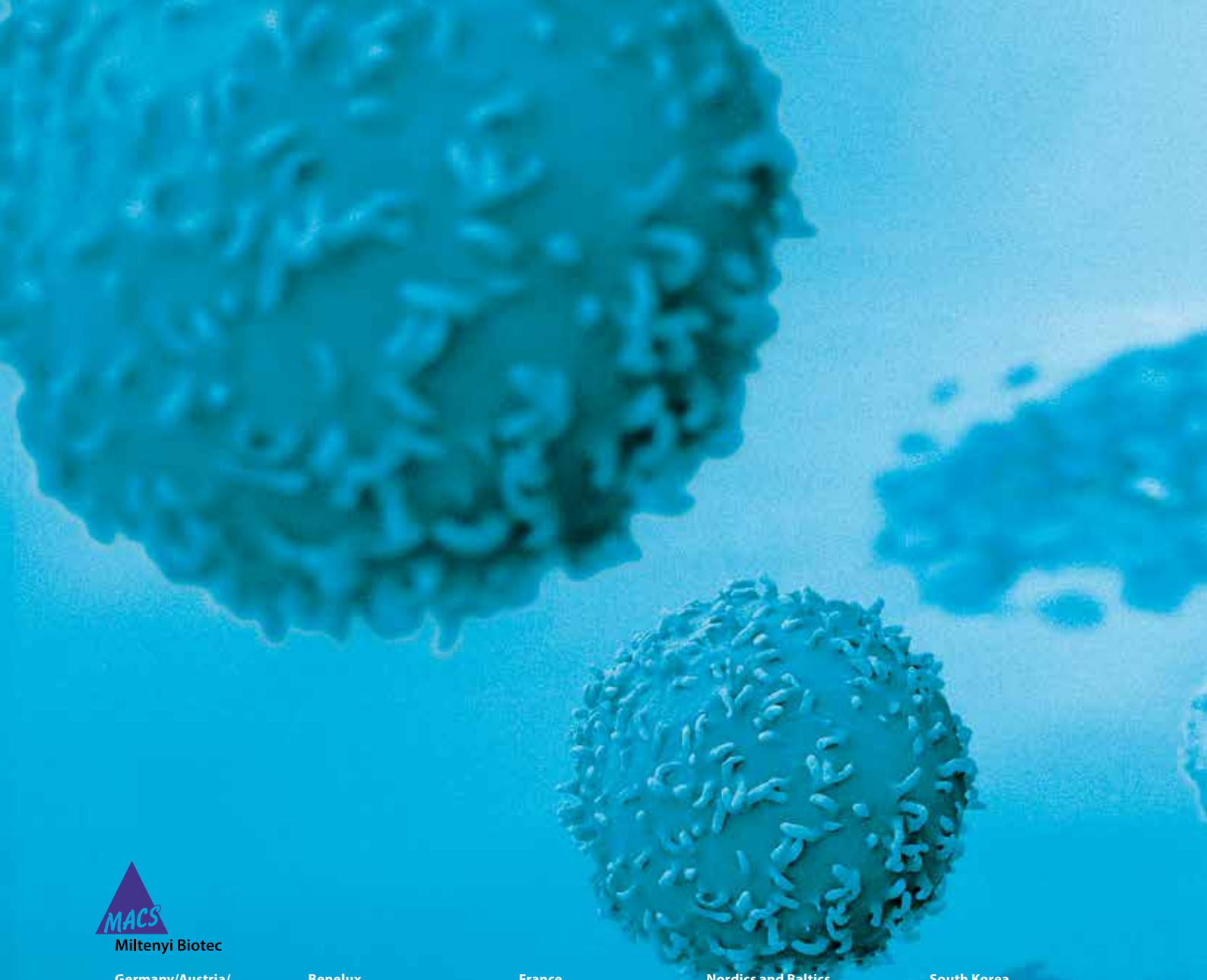
Product	Content	Configuration	Regulatory status	Availability	Order no.
MACS GMP ExpAct Treg Kit	2 vials	2×10^9, ExpAct Treg Beads in 5 mL	[1]	Worldwide[2]	170-076-119
MACS GMP ExpAct Treg Kit for Research Use	2 vials	2×10^9, ExpAct Treg Beads in 5 mL	[4]	USA	130-020-007
MACS GMP ExpAct Treg Kit, CRR-US	2 vials	2×10^9, ExpAct Treg Beads in 5 mL	[3]	USA	200-070-412
TexMACS GMP Medium	1 bag	2000 mL	[1]	Worldwide[2]	170-076-306
MACS GMP Rapamycin	1 vial	200 nM	[1]	Worldwide[2]	170-076-308
MACS GMP Recombinant Human IL-2	1 vial	20 µg	[1]	Worldwide[2]	170-076-113
MACS GMP Expansion Bag	5 bags	up to 100 mL	[1]	Worldwide[2]	170-076-403
MACS GMP Cell Differentiation Bag-100	5 bags	100 mL	[1]	Worldwide[2]	170-076-400
MACS GMP Cell Differentiation Bag-250	5 bags	250 mL	[1]	Worldwide[2]	170-076-401
MACS GMP Cell Differentiation Bag-500	5 bags	500 mL	[1]	Worldwide[2]	170-076-402
MACS GMP Cell Differentiation Bag-1000	5 bags	1000 mL	[1]	Worldwide[2]	170-076-404
MACS GMP Cell Differentiation Bag-3000	5 bags	3000 mL	[1]	Worldwide[2]	170-076-405
MACS GMP CD4-VioBlue	1 vial	5 mL for up to 10^9 total cells	[1]	Worldwide[2]	170-076-504
MACS GMP CD25-PE	1 vial	5 mL for up to 10^9 total cells	[1]	Worldwide[2]	170-076-503
MACS GMP CD25-PE-Biotin	1 vial	5 mL for up to 10^{10} total cells	[1]	Worldwide[2,4]	170-076-505
MACS GMP CD127-APC	1 vial	5 mL for up to 10^9 total cells	[1]	Worldwide[2]	170-076-501
MACS GMP CD45RA-FITC	1 vial	5 mL for up to 10^9 total cells	[1]	Worldwide[2]	170-076-502

[1] MACS GMP Products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications. For regulatory status in the US, please contact your local representative.
[2] For availability in your country please contact your local representative.
[3] In the US, the MACS GMP ExpAct Treg Kit is available for use only under an approved Investigational New Drug (IND) application or Investigation Device Exemption (IDE).
[4] In the US, product is available for research use only.

Products for Treg cell analysis

Product	Configuration	Regulatory status	Order no.
Treg Detection Kit (CD4/CD25/CD127)	25 tests/100 tests	[5]	130-096-076/130-096-082
Treg Detection Kit (CD4/CD25/FoxP3) (PE)	100 tests	[5]	130-094-163
Treg Detection Kit (CD4/CD25/FoxP3) (APC)	100 tests	[5]	130-094-158
Treg Suppression Inspector	5×10^7 MACSiBead Particles in 2.5 mL	[5]	130-092-909

[5] For research purposes only; not for use in humans



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