Miltenyi Biotec

MACS® GMP Fluorescent Antibodies

Facilitating cell manufacturing according to GMP guidelines





JOIN THE FLOW REVOLUTION

Ex vivo processing for cell manufacturing

MACS® GMP Fluorescent Antibodies have been developed for cell labeling. Possible applications could be flow cytometry analysis and subsequent flow sorting of cell populations from heterogeneous human blood products within a GMP manufacturing environment.

- For labeling up to 1×10^9 cells total
- Shelf life of 6 –12 months
- Applicable with the CliniMACS Prodigy® for fully automated labeling of target cells

A wide range of specificities and fluorochromes are available. Moreover, a special custom conjugation program has been introduced that allows the GMP-compliant manufacture of fluorescent antibodies.

Guaranteed

Lot-to-lot consistency
No time-consuming re-evaluation needed.

Regulatory support
Facilitates discussions with your regulatory authorities about, e.g., sterility or endotoxin testing methods.

Extensive stability studies
Rely on consistent protein stability.

Stable supply
Product availability guaranteed.



Figure 1: MACS GMP Fluorescent Antibodies.

Regulatory support (Product-specific documentation)

- Product Information File (PIF)
- Package insert
- Batch-specific Certificate of Analysis (CoA)
- Certificate of Origin (CoO)/TSE (transmissible spongiform encephalopathies)
- USA: Type II Master File (for cross-reference) for selected MACS GMP Fluorochromes

MACS GMP Quality

The success of your cell-based and gene therapy products for clinical research depends on the quality of the raw materials. Consistent high-quality products are essential for reliable and functionally relevant results.

MACS GMP Products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications. As an experienced provider of products for regenerative medicine and tools for cell therapy applications, Miltenyi Biotec caters to our customers' needs with technical and regulatory support and long-term perspectives.

MACS GMP Fluorescent Antibodies are manufactured in our state-of-the-art GMP-certified facility in Teterow, Germany. Manufacturing processes comply with ISO 9001 and ISO 13485. Testing is performed within the quality management system of ISO 13485. The manufacturing and testing of these products is compliant with the requirements laid down in the Ph.Eur. Chapter 5.2.12.

MACS GMP Fluorescent Antibodies are compliant with relevant GMP guidelines and designed following the recommendations of USP <1043> on ancillary materials.

No animal- or human-derived materials were used for the manufacture of this product, unless otherwise stated in the respective Certificate of Origin.

Naive regulatory T cells

Regulatory T (Treg) cells are an interesting target cell population for immunotherapy in a number of different applications. Due to a lack of an exclusive surface marker which clearly distinguishes Treg cells from other T cell subsets, the isolation of an ultra-pure (naive) Treg cell population requires the combination of different surface markers and flow cytometric cell sorting.

To provide the full labeling flexibility, we have developed two different MACS® GMP Fluorescent Antibody panels enabling the detection and GMP-compliant cell sorting of highly pure naive Treg cell populations (table 1 and fig. 3).

Panel A	Panel B
MACS GMP CD25-PE-Biotin	MACS GMP CD25-PE-Biotin
MACS GMP CD4-VioBlue	MACS GMP CD4-VioBlue
MACS GMP CD127-APC	MACS GMP CD127-FITC
MACS GMP CD45RA-FITC	MACS GMP CD45RA-APC

Table 1: MACS GMP Fluorescent Antibody panels for the isolation of Treg cells.



Figure 2: MACS GMP Fluorescent Antibodies are perfectly applicable with the CliniMACS Prodigy® (left) for automated, GMP-compliant labeling and subsequent cell sorting with the MACSQuant® Tyto® Cell Sorter* (right).

* For research use only (RUO) in the USA.

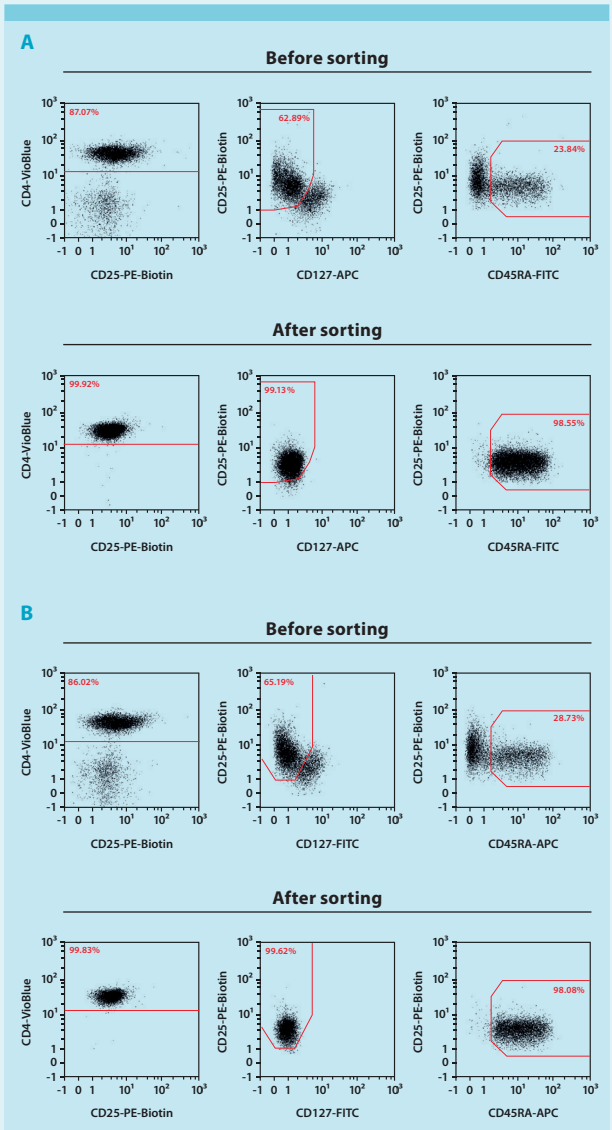


Figure 3: Fluorescent cell sorting of naive Treg cells. CD25+ cells were magnetically pre-enriched from PBMCs by using MACS GMP CD25-PE-Biotin and CliniMACS® Anti-Biotin Reagent. Afterwards, the cells were stained with MACS GMP CD4-VioBlue®, MACS GMP CD127-APC and MACS GMP CD45RA-FITC (Panel A) (A) or with MACS GMP CD4-VioBlue, MACS GMP CD127-FITC and MACS GMP CD45RA-APC (Panel B) (B) and sorted using the MACSQuant Tyto. The fluorescent sort was performed with a sequential sorting strategy: The first sort was conducted with a total cell concentration of ~5×10⁶ cells/mL, followed by a second sort with a cell concentration of ~7×10⁵ cells/mL. The cells were analyzed after magnetic pre-enrichment (before sorting) and after the second cell sorting (after sorting) using a MACSQuant Analyzer 10. The frequency of target cells is depicted in the flow plots.

Antigen-specific T cells

Antigen-specific T cells, e.g., within tumor-infiltrating lymphocytes (TILs), become more and more important in the context of adoptive T cell therapy. A method to identify antigen-specific CD8⁺ T cells is to stimulate the cells with the antigen of interest and thus to trigger the upregulation of activation markers, e.g., CD137 (4-1BB). Based on the marker combination, CD8⁺CD137⁺ target cells can be isolated by flow cytometric cell sorting using the MACSQuant® Tyto® Sorter and MACS® GMP CD8-APC and MACS GMP CD137-PE Fluorescent Antibodies (fig. 4).

After isolation, cells can be expanded using the rapid expansion protocol (REP) on the CliniMACS Prodigy® Platform (fig. 5). This all-in-one cell manufacturing device is a completely closed system and represents the perfect fit for an automated cell expansion approach. It reduces open handling steps and minimizes operator variability, while allowing full flexibility in cell culture conditions.

Visit **www.miltenyibiotec.com/prodigy** to learn more about the CliniMACS Prodigy Platform.

Visit **www.miltenyibiotec.com/tyto** to learn more about the MACSQuant Tyto Cell Sorter.

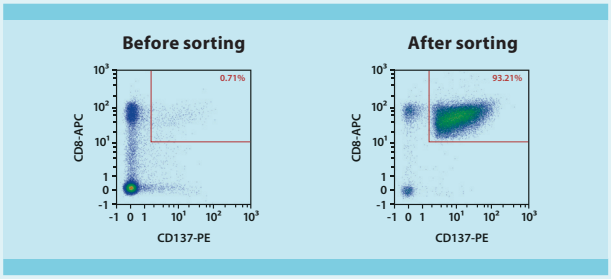


Figure 4: Fluorescent cell sorting of CD8⁺CD137⁺ T cells. Leukapheresis product was stimulated with MACS GMP PepTivator® Peptide Pools HCMV pp65, EBV Select, and AdV5 for 42 hours. Cells were then labeled with MACS GMP CD8-APC and MACS GMP CD137-PE, and sorted by two consecutive sorting cycles. The first sort was conducted with a total cell concentration of 1×10^7 cells/mL, followed by a second sort with a cell concentration of $<1\times10^6$ cells/mL. The frequency of target cells is depicted in the flow plots.

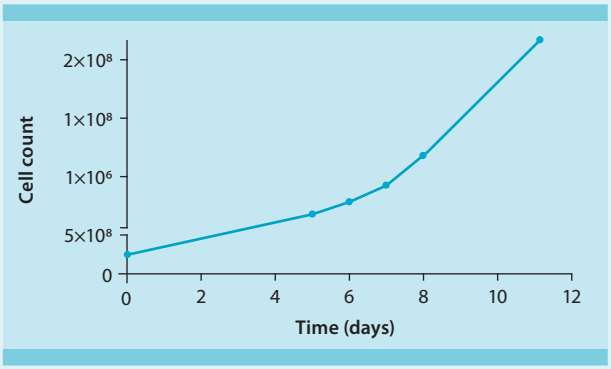


Figure 5: Automated REP of CD8⁺CD137⁺ T cells. 3×10^5 sorted cells were cultured together with 4×10^8 irradiated allogenic feeder cells in TexMACS GMP Medium + 3000 IU/mL MACS GMP IL-2 + 30 ng/mL MACS GMP CD3, pure (clone: OKT3) for 11 days.

Final cell product (after 11 d of expansion)	
Fold expansion	~ 800 fold expansion
Viability	98.9
Final purity	CD8⁺ cells: 95.4%; CD4⁺ cells: 3.4%
% reactive cells*	46% IFN-γ⁺ and 53% CD107a

Table 2: *Expanded cells were restimulated with PepTivator® Peptide Pools HCMV pp65, EBV consensus, and AdV5 for 6 hours. IFN-γ was determined by intracellular staining and CD107a was added during the culture.



Graft engineering

CD34⁺ and CD34⁺/CD133⁺ cells possess the capability to differentiate into cell types of several different tissues and are therefore interesting for various therapeutic uses. CD34 and CD133 enriched stem cell grafts have been employed in autologous and allogenic transplantation both in haploidentical[1-3] as well as HLA-matched settings[4].

To enable the GMP-compliant detection and isolation of CD34⁺/CD133⁺ cells by cell sorting, we have developed the MACS GMP CD34-PE and MACS GMP CD133-APC antibodies.

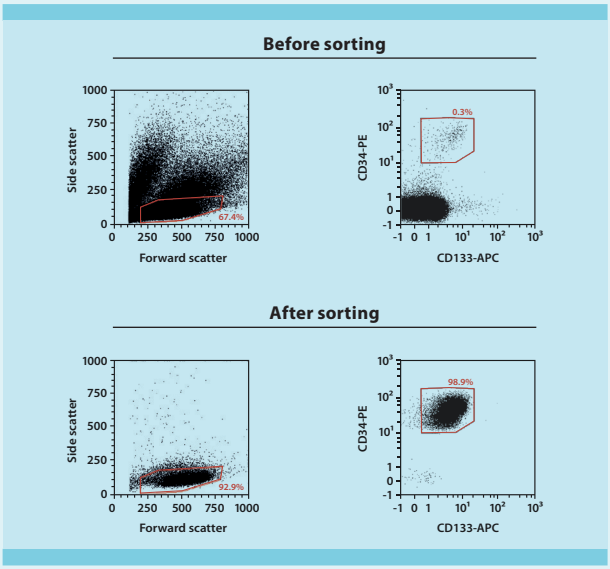


Figure 6: Fluorescent cell sorting of CD34⁺/CD133⁺ hematopoietic stem cells from cryopreserved cord blood. MNCs (mononuclear cells) were isolated from cord blood samples by Ficoll density gradient centrifugation before cryoperservation in StemMACS Cryo-Brew for longterm storage. Upon thawing, cells were labeled with MACS GMP CD34-PE and MACS GMP CD133-APC and sorted using the MACSQuant Tyto Cell Sorter. The fluorescent sort was performed with a sequential sorting strategy: The first sort was conducted with a total cell concentration of 1.5×10^7 cells/mL, followed by a second sort with a cell concentration of 1.8×10^5 cells/mL. Afterwards, cells were analyzed using a MACSQuant Analyzer 10.

References

1. Lang, P. *et al.* (2004) Br. J. Haematol. 124: 72–79.
2. Lang, P. *et al.* (2004) Bone Marrow Transplant. 33: 879–880.
3. Bitan, M. *et al.* (2005) Exp. Hematol. 33: 713–718.
4. Bornhauser, M. *et al.* (2005) Leukemia 19: 161–165.

Portfolio overview

MACS® GMP Fluorescent Antibodies	Order no.
MACS GMP CD127-APC	170-076-501
MACS GMP CD45RA-FITC	170-076-502
MACS GMP CD25-PE	170-076-503
MACS GMP CD4-VioBlue®	170-076-504
MACS GMP CD25-PE-Biotin*	170-076-505
MACS GMP CD4-PerCP	170-076-506
MACS GMP CD8-APC	170-076-507
MACS GMP CD62L-PE	170-076-508
MACS GMP CD34-PE	170-076-509
MACS GMP CD38-APC	170-076-510
MACS GMP CD45RA-APC	170-076-511
MACS GMP CD127-FITC	170-076-512
MACS GMP CD133-APC	170-076-513
MACS GMP CD137-PE	170-076-514

* for magnetic pre-enrichment in combination with the CliniMACS® Anti-Biotin Reagent

Find more information at
www.miltenyibiotec.com/MACS_GMP_Antibodies

For custom development of MACS GMP fluorochromes please contact your local representative.



▸ miltenyibiotec.com/MACS_GMP_antibodies

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In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. The CliniMACS Reagents and Biotin Conjugates are intended for *in vitro* use only and are not designated for therapeutic use or direct infusion into patients. The CliniMACS Reagents in combination with the CliniMACS System are intended to separate human cells. Miltenyi Biotec as the manufacturer of the CliniMACS System does not give any recommendations regarding the use of separated cells for therapeutic purposes and does not make any claims regarding a clinical benefit. For the manufacturing and use of target cells in humans the national legislation and regulations – e.g. for the EU the Directive 2004/23/EC ("human tissues and cells"), or the Directive 2002/98/EC ("human blood and blood components") – must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS System.
In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application, Investigational Device Exemption (IDE), or FDA approval.
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