Miltenyi Biotec

Magnetic enrichment of CD25 positive cells using MACS® GMP CD25-PE-Biotin

Contents

Important note

The present protocols are research protocols, based on laboratory experience. Miltenyi Biotec GmbH cannot and will not accept any liability as to the outcome of procedures. The procedures are for research use only, not for diagnostic or therapeutic purposes.

Note: MACS GMP CD25-PE-Biotin and the CliniMACS Anti-Biotin Reagent are intended for *in vitro* use only and are not supposed to be used for therapeutic application or direct infusion into patients.

1. Description

1.1 Purpose

This protocol describes the process for labeling of cells with MACS GMP CD25-PE-Biotin. MACS GMP CD25-PE-Biotin has been developed for the magnetic pre-enrichment using CliniMACS Anti-Biotin Reagent as second labeling step followed by flow cytometric analysis and flow cytometric sorting of cell populations from human heterogeneous blood products in the clinical setting.

Disclaimer

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 USA, please contact your local representative.

Quality statement

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.

1.2 Reagent and instrument requirements

Product	Order No
MACS GMP CD25-PE-Biotin	# 170-076-505
CliniMACS PBS/EDTA Buffer	# 700-25
CliniMACS Anti-Biotin Reagent	# 173-01
Human serum albumin (HSA) as supplement to CliniMACS PBS/EDTA Buffer, final concentration 0.5%	
Transfer Bags 600 mL	# 130-019-001
Luer/Spike Interconnectors	# 130-018-701
CliniMACS ^{plus} Instrument	# 151-01
CliniMACS Tubing Set TS	# 161-01
Sterile Tubing welder, e.g. Terumo Sterile Connection Device TSCD® SC-201A	
Tube Sealer, e.g. Hematron III	
Flow cytometer, e.g. MACSQuant Analyzer 10 for analysis	#130-096-343
Centrifuge, suitable for bag processing	
Plasma extractor	
Orbital shaker	
Digital Balance	
Tubing Slide Clamps or Scissor Clamps	

Optional for analysis only: Fluorochrome-conjugated Antibodies for flow cytometric analysis, e.g. CD45-VioGreen (#130-096-906), CD4-VioBlue (#130-097-333), CD127-APC (#130-094-890), CD45RA-FITC (#130-092-247), and Propidium Iodide Solution (#130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

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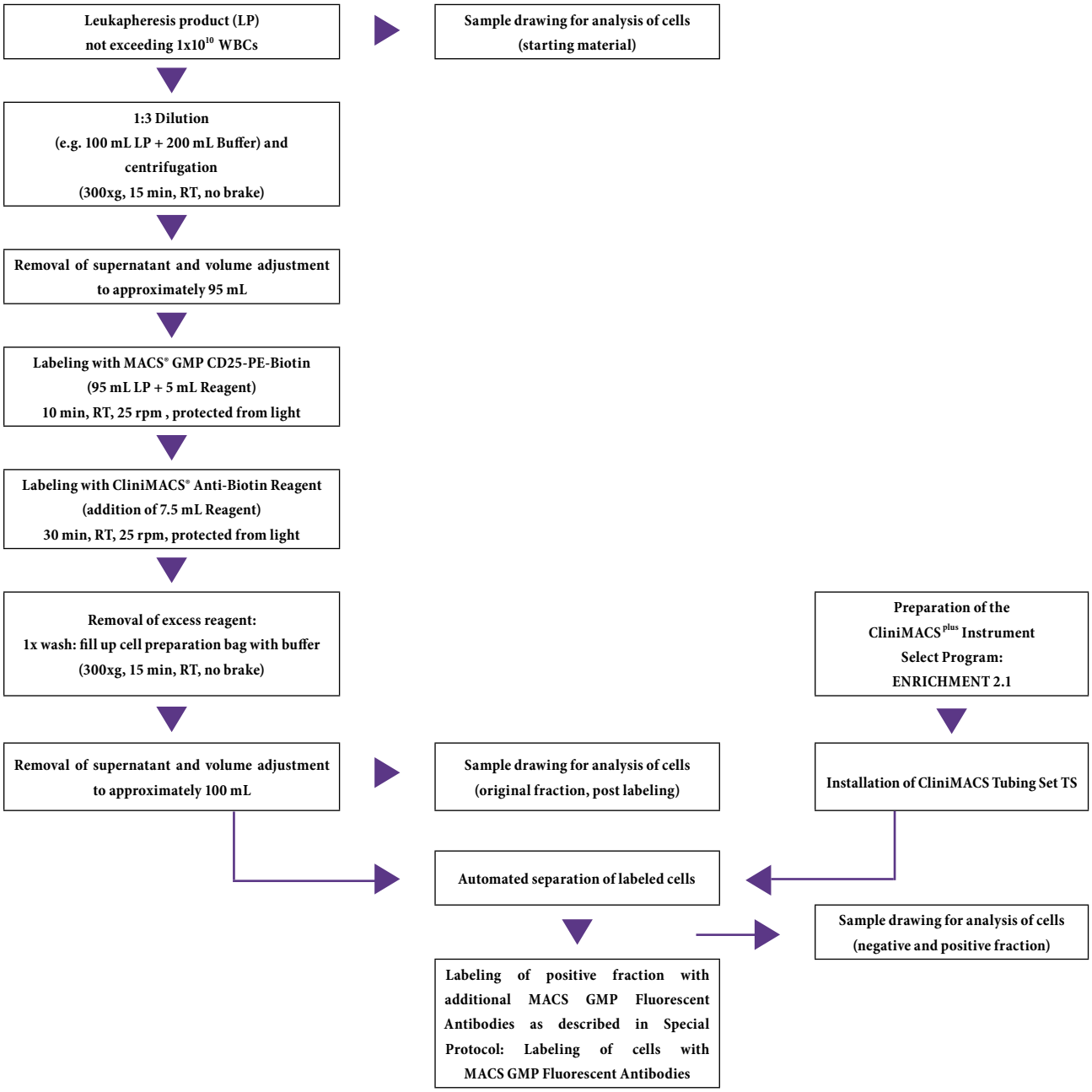
2. Protocol

2.1 Flow chart: Enrichment of CD25$^+$ cells using MACS® GMP CD25-PE-Biotin and CliniMACS® Anti-Biotin Reagent and sample drawing



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2.2 Magnetic labeling of CD25$^+$ cells in bags

Volumes given below are for up to 1×10^{10} total cells. The process requires 2×1 L CliniMACS PBS/EDTA buffer supplemented with HSA to a final concentration of 0.5%.

The cell number (and optionally viability) of the starting material prior to staining has to be determined. Use a small aliquot of the cells for determination of the cell number and viability, e.g. by using the MACSQuant Analyzer 10.

1. Spike the original leukapheresis bag with a Luer/Spike Interconnector and remove a small sample (0.5 mL) using a syringe. Perform cell counts and viability assessment on this sample.
2. Calculate cell number to be used in the experiment, with a maximum of 1×10^{10} total cells.
3. Weigh an empty 600 mL Transfer Bag including a connected Luer/Spike Interconnector and Clamp and label it as 'Cell Preparation Bag'.
4. Transfer correct volume from starting material to the Cell Preparation bag by either syringe transfer or weight via sterile welding of the leukapheresis bag.
5. Dilute the starting material with CliniMACS PBS/EDTA Buffer containing 0.5% HSA by sterile welding a buffer bag and transferring at least three-fold excess of buffer (up to 500 mL).
6. Connect an empty 600 mL Transfer Bag via sterile welding to the Cell Preparation Bag prior to centrifugation and label as 'waste bag 1'.
7. Centrifuge cells at 300×g for 15 minutes at room temperature (+19 °C to +25 °C) without brake.
8. Remove supernatant using the Plasma extractor (taking care not to disturb the pellet), disconnect waste bag 1 and thereafter resuspend the cell pellet completely.
9. Determine the volume of the cell pellet by weighing the Cell Preparation Bag with cells and subtracting the empty bag weight. Add CliniMACS PBS/EDTA Buffer containing 0.5% HSA to a final volume (= weight) of 95 mL.
10. Vortex the MACS GMP CD25-PE-Biotin thoroughly before adding 5 mL (using a syringe) to the Cell Preparation Bag with cells and mix carefully. The total staining volume should now be 100 mL (recommended antibody dilution of 1:20 should be used).

    Note: Vortexing of the MACS GMP CD25-PE-Biotin is important to get the optimal staining performance.
11. Mix the bag thoroughly and incubate for 10 minutes at room temperature (+19 °C to +25 °C) protected from light on an orbital shaker at a maximum of 25 rpm.
12. Add 7.5 mL of the CliniMACS Anti-Biotin Reagent (using a syringe) to the Cell Preparation Bag; the total staining volume should now be 107.5 mL.
13. Mix the bag thoroughly and incubate for 30 minutes at room temperature (+19°C to +25°C) protected from light on an orbital shaker at a maximum of 25 rpm.
14. Wash the cells by adding CliniMACS PBS/EDTA buffer containing 0.5% HSA to a final volume of 500 mL.
15. Connect an empty 600 mL Transfer Bag via sterile welding to the Cell Preparation Bag prior to centrifugation and label as 'waste bag 2'.
16. Centrifuge at 300×g for 15 minutes at room temperature without brake.
17. Remove as much supernatant as possible using the Plasma Extractor (taking care not to disturb the pellet), remove waste bag 2 and resuspend the pellet completely.
18. Determine the volume of the cell pellet by weighing the Cell Preparation Bag with cells and subtracting the empty bag weight. Add CliniMACS PBS/EDTA Buffer containing 0.5% HSA to a final volume (= weight) of approximately 100 mL.
19. Transfer a 0.5 mL sample to a tube for flow cytometric analysis (= original fraction post-labeling). It is recommended to determine at least cell concentration, viability and frequency/number of target cells using additional stainings (see section 2.4).

2.3 Automated separation on the CliniMACS® plus Instrument

All bag handling should be performed in a sterile environment (e.g. laminar flow hood or cleanroom) using aseptic techniques. Connecting tubing with the help of the TSCD may be done outside the laminar flow hood.

1. Weigh an empty 600 mL Transfer Bag (including a connected Luer/Spike Interconnector and Clamp) and label it as 'Cell Collection Bag'. Also weigh the Non-Target Cell bag and Buffer Waste bag included in the CliniMACS® Tubing Set TS.
2. When working in a laminar flow hood using aseptic techniques:
   - Connect the Cell Collection Bag to the luer connector on the CliniMACS Tubing Set TS.
   - Clamp the tube of the tubing set behind the connection for the buffer bag to avoid buffer flowing into the tubing set. Connect a 1 L CliniMACS PBS/EDTA Buffer bag containing 0.5% HSA using the buffer spike of the tubing set.
   - Attach the pre-system filter using the spike of the bubble trap and close the tubing under the bubble trap with a clamp to avoid cells to flow into the tubing set. Connect the Cell Preparation Bag containing the magnetically labeled cells to the CliniMACS Tubing Set TS using the spike of the pre-system filter.
3. Switch on the CliniMACS ^{plus} Instrument and select a suitable program according to the chosen separation strategy. For enrichment of CD25$^+$ cells using MACS GMP CD25-PE-Biotin and the CliniMACS Anti-Biotin Reagent the program ENRICHMENT 2.1 is recommended.

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4. Confirm your choice by pressing 'ENT' and select CliniMACS Tubing Set TS. Enter the Ref. no. of the selected tubing set. The Ref. no. can be found on the product label.
5. Follow the instructions given on the CliniMACS ^{plus} Instrument screen to attach the tubing set to the instrument (if working in a cleanroom, the bags described in point 2 can be connected to the tubing set using Luer/Spike Interconnectors at this point).
6. Ensure that all clamps are opened.
7. Start the automated separation program.
8. After the separation has been finished, disconnect all bags (e.g. using a Tube Sealer such as Hematron III). Determine the weight of the Cell Collection Bag, the Non-Target Cell Bag and Buffer Waste Bag and calculate the weight of each fraction by subtracting the weight of the empty bags.
9. Take a small sample of each fraction for flow cytometric analysis to evaluate the separation performance (see section 2.4).
10. For additional labeling of the magnetically enriched CD25$^+$ cells with MACS GMP Fluorescent Antibodies, take the Cell Collection Bag and relabel this as 'Cell Preparation Bag for Labeling'.
11. Continue with point 6 of the Special Protocol: "Labeling of cells with MACS GMP Fluorescent Antibodies" to add additional MACS GMP Fluorescent Antibodies for cell sorting purposes.

2.4 (Optional) Evaluation and analysis of separation performance

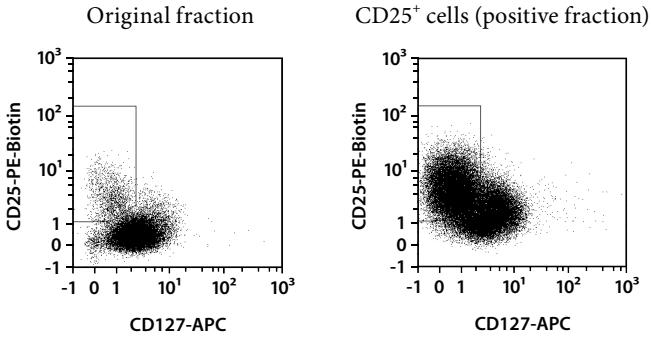
The separation performance of MACS GMP CD25-PE-Biotin on the different cell fractions can be determined by flow cytometry. CD25$^+$ cells can be detected by the PE fluorochrome. Propididum Iodide Solution (#130-093-233) or 7-AAD should be used for flow cytometric exclusion of dead cells.

1. Transfer a small sample of each fraction (e.g. 0.2-0.5 mL) to a tube for flow cytometric analysis. It is recommended to determine at least cell concentration, viability and frequency/number of target cells.
2. Perform additional staining if needed (e.g. CD45-VioGreen™ or PI). For evaluation of target cells we recommend additional staining with CD4-VioBlue (#130-097-333), CD127-APC (#130-094-890), and optionally CD45RA-FITC if desired (#130-092-247).
3. Analyze the samples using e.g. a MACSQuant Analyzer 10.

3. Example of a flow cytometric analysis of MACS® GMP CD25-PE-Biotin magnetically enriched cells

A leukapheresis sample was labeled with MACS® GMP CD25-PE-Biotin and CliniMACS Anti-Biotin Reagent and enriched on a CliniMACS ^{plus} Instrument using the CliniMACS Tubing Set TS (Ref. 161-01) and ENRICHMENT 2.1 Program.

CD4-VioBlue and CD127-APC were added to the original and separated cell fractions. Cells were analyzed by flow cytometry using the MACSQuant Analyzer 10. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. For detailed gating strategy, please refer to the Special Protocol: Labeling of cells with MACS GMP Fluorescent Antibodies.



Warranty

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