Miltenyi Biotec

Handling guidelines

MACS® GMP Cell Culture Bags

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This application note provides practical guidelines for filling, inoculation, incubation, splitting, and harvesting of cells using MACS GMP Cell Culture Bags.
MACS GMP Cell Culture Bags have been developed as valuable tools for cell culturing in a functionally closed system.
The bags are intended for *in vitro* cultivation and expansion of human cells derived from heterogeneous hematologic cell populations.
The gas-permeable MACS GMP Cell Culture Bags are transparent for microscopy and come individually packed, sterile, and tested for endotoxins.

Please carefully read and follow all instructions and safety precautions provided in this document before using the MACS GMP Cell Culture Bags. Because it is not possible to anticipate all conditions of use, additional safety precautions may be required and/or special handling procedures may apply. Please incorporate this information into your individual site safety programs in accordance with applicable standards and regulations.

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

1. Safety precautions

- Do not use cryoprotective agents (e.g. DMSO) since they may compromise bag integrity.
- Do not centrifuge bags.
- Do not use bags for cryopreservation.
- Do not connect bags directly to a patient.
- Disinfection should only be performed with aldehyde- or peroxide-based disinfectant agents. Alcohol or quarternary ammonium complexes may compromise bag integrity. Ingress of disinfectant agents my harm the cultured cells.
- Careful disinfection of connectors and septum before and after usage is recommended.
- Aseptic working procedures must be applied for unpacking, filling, sampling, or harvesting.
- Air should be carefully removed from the MACS GMP Cell Culture Bag when filling. It is especially important to remove air when filling the first compartment of the MACS GMP Cell Expansion Bag in order to prevent premature opening of the first seal.
- Do not write or place labels on the surface of the bag.
- The validation and quality control of incubators should be in compliance with your institution's equipment maintenance protocol.
- Never stack or overlap cell culture bags in the incubator.

2. MACS® GMP Cell Culture Bags – types and components

MACS® GMP Cell Culture Bags consist of a polyolefine. The bag material is gas-permeable and transparent for microscopy. The bags are sterilized by an e-beam sterilization method. Both sides of the inner surface are identical and not coated. All bags are equipped with a PVC tubing (2.5/4.1 mm inner/outer diameter) and suitable for sterile docking (according to the instructions for use of tube welding devices). The tubing has a female luer lock with a male luer lock cap. Female luer locks allow for fluid transfer via a transfer device with a male luer lock. The luer lock prevents accidental disconnection during fluid transfer. The additional septum port provides access to transfer fluids with a needle and syringe. This port is for single-use only.

Two types of MACS GMP Cell Culture Bags are available. **MACS GMP Cell Differentiation Bags** consist of one culture chamber. Five sizes are available for nominal volumes of 100, 250, 500, 1000, and 3000 mL. **MACS GMP Cell Expansion Bags** have a compartimentalized culture chamber with easy-to-open seals. This allows for expandable volumes from 8 mL to up to 100 mL. Therefore, it is not necessary to transfer the culture into a new vessel when cell numbers increase during culture periods. This feature reduces the risk of contamination.

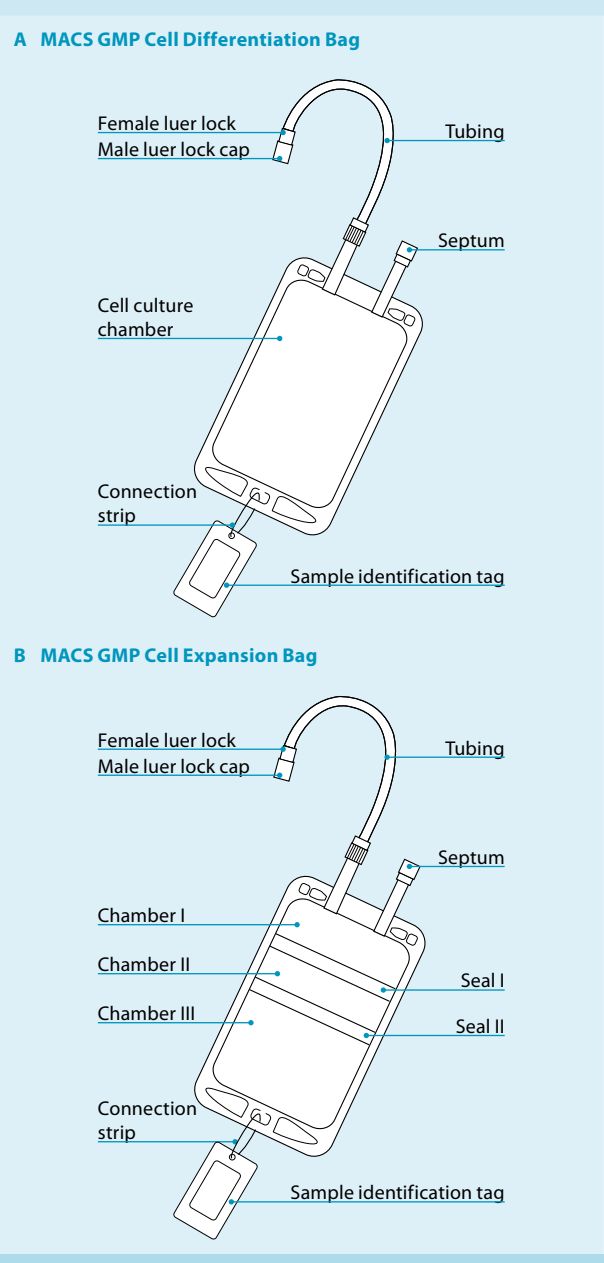

Figure 1: MACS GMP Cell Differentiation Bag (A) and MACS GMP Cell Expansion Bag (B) with two seals for explandable culture volumes.

3. Preparing medium

Prepare a sufficient amount of medium according to your laboratory protocols.

4. Fluid transfer into bags

Aseptic working procedures must be applied for unpacking, filling, sampling, or harvesting.
Air should be carefully removed from the MACS GMP Cell Culture Bag when filling. It is especially important to remove air when filling the first compartment of the MACS GMP Cell Expansion Bag in order to prevent premature opening of the first seal.
Careful disinfection of connectors and septum before and after usage is recommended.

4.1 Small-volume fluid transfer

For the aseptic transfer of small volumes (20–130 mL) into MACS GMP Cell Culture Bags either the female luer lock or the septum port can be used.

Figure 2: MACS GMP Cell Differentiation Bags are equipped with a septum port (left) and a tubing with female luer lock and cap (right).

4.1.1 Small-volume fluid transfer via female luer lock

1. Clamp the tubing of the MACS GMP Cell Culture Bag.

Figure 3: Clamped tubing.

2. Remove the cap from the female luer lock.
3. Attach medium-filled syringe to the female luer lock, open the clamp, and inject medium while holding the bag in an upright position.

Figure 4: Medium-filled syringe attached to the female luer lock of the MACS GMP Cell Culture Bag.

4. Push a small amount of air through the tubing to ensure that no medium remains in the tubing.
5. Holding the bag in an upright position, remove as much excess air from the bag as possible by pulling the plunger of the syringe. Tap the bag and remove any remaining air until medium reaches the tubing.
6. Clamp the tubing as close to the bag as possible.
7. Using a dielectric sealer, seal the tubing three times.
   Note: Seal the tubing close to the luer lock to ensure that adequate tubing is left for future sterile docking connections, if needed.

Figure 5: Sealing of the tubing with a dielectric sealer.

8. Remove the tubing by cutting through the central seal.

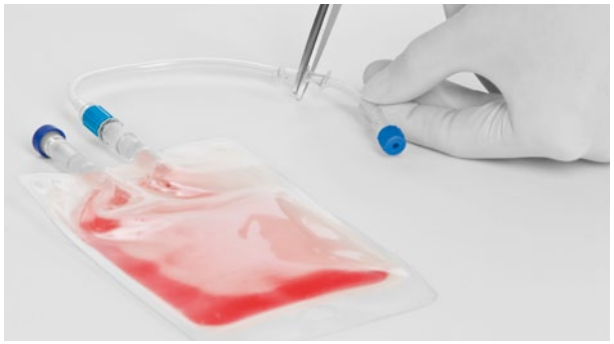

Figure 6: Cutting through the central seal.

4.1.2 Small-volume fluid transfer via the septum port

It is recommended to reserve the septum port for future splitting of cell cultures.

1. Swab the septum port with an appropriate disinfectant.
2. Insert medium-filled syringe with needle straight into the septum port and inject medium while holding bag in an upright position.

Figure 7: Medium-filled syringe attached to the septum port.

3. Push a small amount of air through the port to ensure that no medium remains in the port.
4. With the bag in an upright position, remove as much excess air from the bag as possible by pulling the plunger of the syringe. Tap the bag and remove any remaining air until medium reaches the port.
5. Remove the syringe/needle and swab the septum port with an appropriate disinfectant.

4.2 Instructions for filling MACS® GMP Cell Expansion Bags

The MACS GMP Cell Expansion Bag has three compartments with easy-to-open seals to allow for an expandable culture volume. Make sure to carefully remove air from the bag when filling the first compartment to prevent premature opening of the first seal.

Figure 8: Consecutive filling of the compartments of the MACS GMP Cell Expansion Bag.

To open the next compartment, transfer additional medium as necessary and apply gentle pressure to the fluid in the direction of the seal until it is fully opened.

Figure 9: Opening a seal of the MACS GMP Cell Expansion Bag.

4.3 Large-volume fluid transfer from medium bags into MACS® GMP Cell Culture Bags

Large volumes (100–1200 mL) of fluids can be aseptically transferred from medium bags into MACS GMP Cell Culture Bags. Medium bags can be connected to the MACS GMP Cell Culture Bags using different methods, depending on the connectors available at the medium bag.

Method 1: Aseptically connect the male luer lock end of a spike and male luer lock set to the female luer lock at the tubing of the MACS GMP Cell Culture Bag. Aseptically insert the spike end into the spike port of the medium bag.

Figure 10: Spike and male luer lock set attached to the female luer lock of the MACS GMP Cell Differentiation Bag.

Method 2: Aseptically connect the needle end of a spike and needle set to the septum port of the MACS GMP Cell Culture Bag. Aseptically insert the spike end into the spike port of the medium bag.

Figure 11: Spike and needle set attached to the septum port of the MACS GMP Cell Differentiation Bag.

Method 3: Use a sterile docking device to connect the tubing of the MACS GMP Cell Culture Bag to a tubing of the medium bag.
It is recommended to reserve the septum port for future splitting of cell cultures.

1. Clamp the tubing of the MACS GMP Cell Culture Bag.
2. Connect the medium bag to the MACS GMP Cell Culture Bag utilizing the appropriate method.
3. Tare a top loading balance with the empty cell culture bag in place. The tubing set should not be included in the weight measurement.
   Note: If weighing a culture bag larger than the balance tray, the cell culture bag can be supported with a tray/cardboard larger than the bag.
4. Open the clamp.
5. Allow the culture medium to drain into the cell culture bag until the required volume is achieved, as indicated on the balance.

Figure 12: Fluid transfer from MACS GMP Cell Culture Bag into new receiving bag.

6. Close the clamp on the cell culture bag.
7. Using a dielectric sealer, seal the used tubing three times and disconnect by cutting through the central seal (see figures 5 and 6).
   Note: Seal tubing close to the spike to ensure adequate tubing is left for future sterile docking connections, if needed.

5. Inoculation, incubation, and cell culture sampling

5.1 Inoculation

For the transfer of cells into the MACS GMP Cell Culture Bags, follow the procedures outlined in section 4.1.1 or 4.1.2 for small-volume fluid transfer. Inoculation via the septum port is recommended.

5.2 Incubation

All MACS GMP Cell Culture Bags are delivered with a set of plastic sample identification tags, which can be attached to the bag by a connection strip.

Note: Do not write or place labels on the surface of the bag.
Note: The validation and quality control of incubators should be in compliance with your institution's equipment maintenance protocol.

1. Hermetically seal the tubing of the cell culture bag leaving adequate tubing for future sterile connections.
2. Place the Cell Culture Bags horizontally on an incubator shelf. Ensure that the incubator shelf is level (on parallel shelf guides) and that the cell culture bag is completely flat to provide optimal cell expansion. The integral tubing set can be laid next to or on top of each Cell Culture Bag.
   Note: Never stack or overlap cell culture bags in the incubator.
3. To allow for optimal gas exchange, the surface of the cell culture bag must not be obstructed.

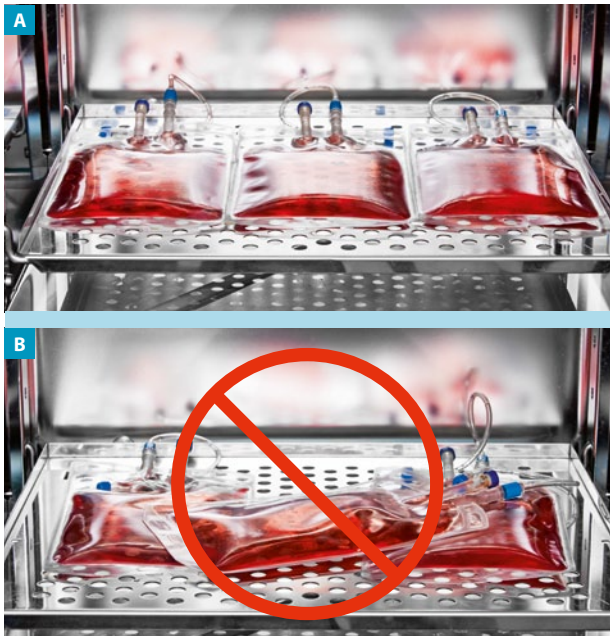

Figure 13: Placement of cell culture bags in an incubator (correct: A, incorrect: B).

5.3 Cell culture sampling

1. Insert a needle and syringe into the septum port of the MACS GMP Cell Culture Bag.
2. Invert cell culture bag. Fill and empty syringe several times with the cell suspension by pulling and pushing the plunger of the syringe. This ensures the removal of a statistically valid sample.
3. Remove required sample.
   Note: Disinfect the septum port before and after usage with an appropriate disinfectant. Disinfection should only be performed with aldehyde- or peroxide-based disinfectant agents. Alcohol or quarternary ammonium complexes may compromise bag integrity. Ingress of disinfectant agents my harm the cultured cells.

6 Cell culture splitting with sterile connection device (SCD)

Split or transfer cell culture from MACS GMP Cell Culture Bags into additional cell culture bags to reduce cell density and add fresh nutrients for further cell expansion as follows:

1. Clamp the tubing of the MACS GMP Cell Culture Bags.
2. Using your SCD connect the new receiving MACS GMP Cell Culture Bag to the source MACS GMP Cell Culture Bag in a sterile way using the tubing.

Figure 14: MACS GMP Cell Culture Bag and new receiving bag installed in an SCD.

Figure 15: MACS GMP Cell Culture Bag and new receiving bag after sterile connection.

3. Place the new receiving cell culture bag on a top loading balance. Tare the balance. The tubing should not be included in the weight measurement.
4. Invert the source cell culture bag by hanging using the hanger hole.
5. Ensure that the balance reads zero.
6. Open the clamps on both cell culture bags. Allow the cell culture to transfer into the receiving cell culture bag until the required volume is achieved (see figure 12).
7. Stop the transfer by clamping the tubing.
8. Hermetically seal and disconnect the tubing between the receiving and the source cell culture bags. Leave enough tubing for future sterile connections (see figures 5 and 6).
9. Repeat steps 1–8 for each additional culture bag required.
10. Connect the new receiving cell culture bag to the medium bag. To transfer medium, follow the procedure described in section 4.3 on large-volume fluid transfer.

7 Cell culture harvest

7.1 Small-volume cell culture harvest via female luer lock

1. Clamp the tubing of the MACS GMP Cell Culture Bag.
2. Gently mix contents of the cell culture bag.
3. Attach a syringe to the female luer lock on the cell culture bag.
4. Invert cell culture bag and open clamp. Remove cell culture suspension by pulling the plunger of the syringe until syringe is filled.

Figure 16: Empty syringe attached to female luer lock of a MACS GMP Cell Culture Bag.

5. Close clamp and disconnect the syringe.
6. Transfer cell culture suspension via the syringe into a bag or tube suitable for centrifugation.

7. Steps 1–6 can be repeated several times until the entire culture volume has been harvested. The cell culture bag can be rinsed by injecting additional wash solution and transferring the solution into the harvested cell suspension.
8. Centrifugation should be performed according to your institution's protocols.

7.2 Small-volume cell culture harvest via septum port

1. Clamp the tubing of the MACS GMP Cell Culture Bag.
2. Gently mix contents of the cell culture bag.
3. Disinfect the septum port with an appropriate disinfectant.
4. Aseptically insert syringe and needle straight into the septum port.
5. Invert cell culture bag and open clamp.
6. Pull the plunger of the syringe to remove the cell culture suspension.

Figure 17: Empty syringe attached to septum port of a MACS GMP Cell Culture Bag.

7. Close the clamp, remove syringe and needle, and transfer the cell culture suspension into a bag or tube suitable for centrifugation.
8. Repeat steps 1–7 until the entire culture volume has been harvested. The cell culture bag can be rinsed by injecting additional wash solution and transferring the solution into the harvested cell suspension.
9. Centrifugation should be performed according to your institution's protocols.

7.3 Large-volume cell culture harvest

1. Gently mix the contents of the MACS GMP Cell Culture Bag to resuspend the cells.
2. Obtain the required number of 600 mL transfer bags, into which the cell culture from the source culture will be transferred. A maximum volume of 500 mL can be placed into a transfer bag.
3. Using an SCD, connect the tubing of the transfer bag to the tubing of the cell culture bag.
4. Open the tubing seal, drain the culture suspension into the transfer bag.
   Note: A top loading balance can be used to measure the transferred volume.
5. Hermetically seal tubing and disconnect cell culture bag from transfer bag. Leave enough tubing on the cell culture bag to allow for future sterile connection.
6. Repeat steps 3–5 until the cell culture bag is empty. The cell culture bag can be rinsed by injecting additional wash solution and transferring the solution to a transfer bag as described.
7. Using the SCD, connect each transfer bag containing the cell culture suspension to the tubing of a new transfer bag for collecting waste medium. Pellet the suspension in the transfer bag by centrifugation according to your institution's protocol (e.g. 800×g for 10 minutes).
8. Carefully remove transfer bags from centrifuge.
9. Place transfer bag containing the cell pellet in a plasma extractor.
10. Open the tubing seal between the waste bag and the transfer bag containing the cell pellet.
11. Slowly release the handle of the plasma extractor and allow the supernatant to drain into the waste bag.
12. Seal tubing and disconnect transfer bag containing the cell pellet from the waste bag.
13. If a cell wash is required, the transfer bag containing the cell pellet can be connected to the tubing of a bag containing wash buffer, using an SCD. Transfer wash buffer to the transfer bag. Repeat steps 7–12 to pellet the cells.
14. Cells are now ready for final processing according to your institution's protocol.

8. Literature

1 Garritsen, H.S. *et al.* (2010) Efficient generation of clinical-grade genetically modified dendritic cells for presentation of multiple tumor-associated proteins. Transfusion 50(4): 831–42.

2 Macke, L. *et al.* (2010) Evaluating maturation and genetic modification of human dendritic cells in a new polyolefin cell culture bag system. Transfusion 50(4): 843–55.

9. Technical specifications of MACS® GMP Cell Culture Bags

Upon determination of your optimal culture volume, select the appropriate bag size based on the specified minimum fill volume.
The thickness of the fluid layer within the MACS GMP Cell Culture Bag has an impact on gas exchange during the culture process. Gas exchange occurs across the entire surface area of the culture bag. Use of too high a fill volume results in an increased fluid level that may impede gas and nutrient diffusion. Use of a fill volume lower than recommended may result in an accumulation of fluid in one area of the bag (pooling), which may also impede gas and nutrient diffusion and potentially lead to decreased cell expansion.
If the volume required for cell culture is between two culture bag sizes, it is recommended to use the larger bag size. The larger bag allows optimal exposure of the culture volume along the surface area of the bag.

Product	Order no.	Content	Nominal fill volume (mL)	Minimal fill volume (mL)	Fill volume per 1 cm layer thickness (mL)	Average weight (g)	Area (cm^2)
MACS GMP Cell Differentiation Bag – 100	170-076-400	5 bags	100	20	55	14 +/– 1	116
MACS GMP Cell Differentiation Bag – 250	170-076-401	5 bags	250	30	130	15 +/–1	156
MACS GMP Cell Differentiation Bag – 500	170-076-402	5 bags	500	50	250	18 +/– 0.5	230
MACS GMP Cell Differentiation Bag – 1000	170-076-404	5 bags	1000	120	380	25 +/– 1	337
MACS GMP Cell Differentiation Bag – 3000	170-076-405	5 bags	3000	220	1060	40 +/– 1	672
MACS GMP Cell Expansion Bag	170-076-403	5 bags	up to 100	8/16/20	10/20/40	13 +/– 1	27/59/116

Related items	Order no.	Content
Transfer Bag 150 mL*	130-018-301	5 bags
Transfer Bag 600 mL*	130-019-001	5 bags
Transfer Bag 1000 mL*	130-018-001	5 bags
Luer/Spike Interconnector*	130-018-701	5 pieces

*Availability: Europe, other[1), 2)]
[1)] For availbility in your country please contact your local representative.
[2)] Not available for use in the USA.

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