StemRD
EMPOWERING STEM CELL R&D

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MesenGro® Chemically Defined MSC Medium

Product Overview

*The MesenGro® Chemically Defined MSC Medium (for simplicity, referred to as "**MesenGro**" in this document) has been designed for the maximum expansion of human mesenchymal stem cells (MSCs) derived from bone marrow, cord blood, or adipose tissue.*

MesenGro is chemically defined as its components are either chemically synthesized, recombinantly produced and purified. It can be considered xeno-free as none of its ingredients are directly derived from non-human animals. MesenGro further offers the convenience of no plate-coating if used with certain tissue culture vessels (see below), saving cost of coating materials and time needed for coating.

Content and Storage of the Product

MesenGro (Catalog No. MGro-500) contains two components:

	Size	Storage	Shelf Life
MesenGro Basal Medium	1 x 450mL	2 to 8°C. Protect from light	6 months
MesenGro Supplement	1 x 50mL	-80°C. Protect from light	6 months

Additional key reagents required or suggested

- L-Glutamine, GlutaMAX (GIBCO), or glutaGro (Mediatch 25-015-CI)
- Without plate-coating: BD **Primaria**™ tissue culture flasks (e.g., 25 cm^2 tissue culture flasks, BD cat# 353808), or Corning **CellBIND**® tissue culture flasks (e.g., 25 cm^2 tissue culture flasks, Corning cat# 3289)
- With plate-coating: Fibronectin (StemRD cat# Fibro-10 or equivalent)
- Trypsin 0.05%/0.53 mM EDTA (e.g. Mediatech #25-051-CI) or TrypLE™ Express (GIBCO #12604), or equivalent

Medium Preparation

1. Thaw MesenGro Supplement. It is recommended to thaw overnight at 2 to 8°C. Thawed material can be aliquoted and stored at -80°C, but further freeze-thaw cycles should be avoided.

2. To make 100 mL MesenGro complete medium, aseptically add 10 mL MesenGro Supplement to 90 mL MesenGro Basal Medium, and then aseptically add 1 mL of 200 mM L-Glutamine.

3. Antibiotics such as the Pen-Strep solution can also be added to the complete medium if desired. We recommend the final concentrations of 100 units/mL Penicillin and 100 microgram/mL Streptomycin.

Once made, MesenGro complete medium (basal, supplement and L-glutamine) is stable for 1 month when stored in the dark at 2 to 8°C.

Adaptation of MSCs previously cultured in other media:

MSCs cultured in serum-containing or serum-free media can be quickly and easily adapted into MesenGro. In most cases, a one-step transition into MesenGro complete medium is sufficient. If so desired, step-wise adaptation with a gradual increase of the amount of MesenGro (e.g. 20%, 40%, etc) can also be performed.

Recovery of Cryopreserved Human MSCs:

Frozen human MSCs, regardless of what medium was used to grow or freeze them before, can be easily and quickly adapted into MesenGro. A one-step transition into MesenGro medium as outlined below is generally sufficient for cells that have been grown and frozen in serum-containing or serum-free media.

1. Rapidly thaw frozen vial of cells in a 37°C water bath.

2. Transfer the cells into a 50 mL conical tube.

3. For every 1 mL of cell suspension, add 10 mL of pre-warmed (37°C) MesenGro complete medium in a drop-wise manner while gently swirling the tube.

4. Plate the contents of the conical tube into a tissue culture flask or multiple wells of a tissue culture plate. Alternatively, cells can also be centrifuged at 250 x g (~1200 rpm) for 10 minutes, resuspended in MesenGro complete medium and then plated.

5. Incubate at 36 to 38°C in a humidified atmosphere containing 4 to 6% CO_2.

6. After 24 hrs, discard old medium and feed cells with new medium.

For research use only. Not intended for human diagnostic or therapeutic uses.

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7. Maintain the culture by changing medium every 2 days until cell passaging is needed.

Passaging MSCs in MesenGro:

Coating of cell culture vessels is NOT necessary when MesenGro is used with certain tissue culture vessels, for example, the BD **Primaria™** tissue culture products (the 25 cm^2 tissue culture flasks, BD cat# 353808) or the Corning **CellBIND®** tissue culture products. Additional brands and models of tissue culture vessels are currently under evaluation. For tissue culture vessels that have not been evaluated or for those found to be incompatible with the no-coating procedure, we recommend coating with Fibronectin (StemRD cat# Fibro-10) at 0.5 – 1 microgram / cm^2 surface area for 1 hour at 37°C, followed by plating cells in MesenGro.

1. Visually inspect the stock culture (growing in MesenGro or other medium formulations) under the microscope and confirm that the cells are ready to be sub-passaged. To maintain the optimal growth potential of MSCs, it is recommended that the cells be passaged when they reach 70% confluency. If the MSC culture is allowed to reach a confluency of 80% or higher, they may stop proliferating after the passage. **Therefore, IT IS CRUCIAL NOT TO GROW CELLS OVER 80% CONFLENCY under any circumstances!**

2. Pre-warm 0.05% Trypsin/0.53 mM EDTA (e.g. MediaTech 25-051-CI) or TrypLE™ Express (GIBCO 12604) and MesenGro complete medium to 37°C before use.

3. Remove spent (old) medium from the flask using a pipet and discard.

4. Optional: Wash tissue culture surface with DPBS, remove and discard.

5. Add the 0.05% Trypsin/EDTA solution or TrypLE™ Express to the flask, tilt flask to cover all the cells. We recommend detaching the cells at room temperature.

	Individual well of a 6-well plate (~10 cm^2)	T25 flask (25 cm^2)
Volume of Trypsin or TrypLE Express	0.5 mL	1 mL

6. Observe the cells under a microscope. When cells start to detach, gently tap the side of the vessel to help loosen the remaining cells. The time required for the cells to detach should be 1 to 3 minutes if the cells have been cultured in MesenGro. Cells grown in serum-containing media will require longer incubation time to detach.

Once all the cells have detached, proceed quickly to the following step. Do not leave cells in Trypsin or TrypLE™ Express for an extended amount of time after the cells have detached, as this will adversely affect the growth of MSCs.

7. Upon cell detachment, add MesenGro at twice the volume of the trypsin solution used (e.g. 2 mL for a T25 flask). Collect the cell suspension in a sterile 15 mL conical tube.

Optional: If 0.05% Trypsin/EDTA solution is used to detach the cells, it is desirable to use the Soybean Trypsin Inhibitor to neutralize trypsin. This is especially necessary if the washing step (step 8) cannot be processed quickly (within 10 minutes). [This is because serum-free media formulations like MesenGro does not contain antitrypsin, a component found in animal serum. Unless removed quickly by centrifugation, trypsin can damage the cells and affect growth.] If TrypLE Express is used to detach the cells, then it is not necessary to neutralize it according to its manufacture.

8. Centrifuge cells at 1200 rpm (250 x g) for 10 minutes.

9. Aspire and discard as much supernatant as possible. Resuspend cells in pre-warmed MesenGro complete medium. Take an aliquot from the cell suspension for cell counting.

10. Place cells into tissue culture vessels at a density of **5 x10^3 cells/cm^2**. Tilt the vessel a few times to ensure even distribution of cell suspension.

	Individual well of a 6-well plate (~10 cm^2)	T25 flask (25 cm^2)
Seeding cell number	5 x 10^4	1.25 x 10^5

11. Incubate at 37°C in a humidified atmosphere of 4 to 6% CO_2.

12. Replace culture medium every 2 days with fresh, pre-warmed MesenGro complete medium.

13. Pass cells when cell confluency reaches 70% (typically at 3 day intervals).

Cryopreservation of Cells in MesenGro

1. Prepare cryopreservation solution by supplementing MesenGro Basal Medium with 10% MesenGro Supplement and 10% Dimethyl Sulfoxide (DMSO).

2. Pellet cells by centrifugation, resuspend cells in cryopreservation solution to 1.0x10^6 cells/mL, and transfer to cryovials.

3. Place cryovials in a freezing container (e.g. Nalgene 5100-0001) and place in a -80°C freezer overnight.

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4. Transfer cryovials to liquid nitrogen for long-term storage.