

## MAGNOLIA

Culture medium for human mesenchymal stem cells.

A defined, serum free, animal component free medium, developed to support hMSC's growth.



### BENEFITS

MAGNOLIA medium was developed for growth and expansion of human mesenchymal stem cells (hMSC) isolated from umbilical cord. This mesenchymal stem cell medium is available as ready-to-use and consist of a basal medium and one vial of Supplement Mixture.

MAGNOLIA is suitable for long-term growth of hMSC while maintaining their stem cell character.

MAGNOLIA offers

**Simplicity:** Ideal from bench to manufacturing scale.

**Serum-free and animal component free:** Reduces the risk for animal derived contaminants.

**Fully defined:** It doesn't contain hydrolysates; thus, process variability is reduced. It contains recombinant proteins.

**Antibiotic-free:** No risk of antibiotic traces, hidden contamination and endotoxins.

### SPECIFICATION

|                 |   |
|-----------------|---|
| Regulatory      | Free of serum, animal components, hydrolysate, antibiotics w/ recombinant proteins w/ L-Glutamine |
| Available Form  | Liquid, GMP or GMP or non-GMP with or w/o phenolred   |
| Cell Line       | Human Mesenchymal Stem Cells  |
| Medium storage  | 2-8 °C, protect from light  |
| Supplement Mix  | -20 °C, frozen until use  |
| Complete medium | Stable up 3 months  |
| Application     | Research / manufacturing  |

# APPLICATION

## MEDIUM PREPARATION

- The frozen Supplement Mix should be thawed at 2-8 °C or at room temperature, but not at 37 °C. Avoid repeated freeze-thaw cycles.
- For completing the medium, add 1 mL of Supplement Mix into 500 mL of MAGNOLIA basal medium. The completed medium can be stored at 2-8 °C in dark, up to month.
- MAGNOLIA basal medium contains L-Glutamine and it is with or without phenol red.
- MAGNOLIA medium is serum free, animal component free and xeno-free.
- Medium can be used serum free. However, during hMSC isolation and by special applications, hMSC might require serum. In that case you might supplement the medium with 2 % human AB serum (Human serum, Type AB, male, HUM-3B, Capricorn Scientific).

## TREATMENT OF T-FLASKS

There are three different ways of culturing hMSC in T-flask, depending on serum use:

1. Cultivation of cells in 2 % human AB serum containing medium. In this case no special treatment of T-flasks needed by you. Just select a ready to use treated T-flask (TC, Greiner Bio-One, Austria).
2. Cultivation of cells in serum free medium and coating of T-flask with serum containing medium. In this case add to DMEM medium 10 % human AB serum. Put the medium into the treated T-flask and incubate the medium and flask (w/o cells) in incubator for 3 hours. Thereafter, takeout the medium and wash the T-flask with DPBS for 2 times. Don't let the plates dry. Thereafter, put into the T-flask MAGNOLIA medium with Supplement Mix but without serum and inoculate with cells. Repeat this in every passaging of cells.
3. Cultivation of cells in serum free medium and coating of T-flask with a Fibronectin solution. In this case, obtain a Fibronectin solution, coat the treated T-flasks with the solution according to the instruction manual of manufacturer. Thereafter, add into the T-flask MAGNOLIA medium with Supplement Mix, but without serum and inoculate with cells.

## CELL SUB-CULTURING PROCEDURE

MAGNOLIA medium was developed for optimal proliferation of hMSC from variety of sources.

- Add fresh culture medium to a new flask as shown in the table below and place the flask into the incubator for equilibration (7 % CO<sub>2</sub>, 36.7 °C, humidified environment).
- Remove culture medium from T-flask with grown cells and wash the cells once with pre-warmed DPBS w/o Ca, w/o Mg.
- Add to the cells 0.25 % Trypsin-EDTA solution (1 mL for T25 flask, 3 mL for T75 flask).
- Incubate for 2-3 minutes at 36.7 °C and verify cell dissociation by eyes.
- Following detachment, add 9 mL DPBS to the cells, and collect cell suspension in a falcon tube.
- Centrifuge cells (4 min, 400 g, RT), discard supernatant.
- Resuspend the cell pellet in 1 mL pre-warmed supplemented MAGNOLIA medium and count the cells.
- Inoculate cells into the new culture vessel, which was equilibrated with medium previously. Seeding densities are indicated in the table below.

- Incubate the cells (7 % CO<sub>2</sub>, 36.7 °C, humidified environment).
- Cells will be splitted 3 or 4 days after inoculation depending on donor. If cells have not reached a confluency of minimum 80 % at day 3, then refresh the cells with pre-warmed supplemented MAGNOLIA medium and culture the cells one more day. Latest at day 4 after inoculation, split the cells regardless of confluency.
- Optimal confluency for cell split is between 80 and 95 %. Avoid 100% confluency, as it induces cellular senescence and maturation.

| Culture vessels              | 6-well plate                                | T25-Flask                                   | T75-Flask                                   |
|------------------------------|---|---|---|
| Surface area cm <sup>2</sup> | 9.6   | 25  | 75  |
| Working Volume               | 4 mL  | 6 mL  | 15 mL                                       |
| Seeding Densities            | 5.0 x 10 <sup>3</sup> cell/ cm <sup>2</sup> | 5.0 x 10 <sup>3</sup> cell/ cm <sup>2</sup> | 5.0 x 10 <sup>3</sup> cell/ cm <sup>2</sup> |

**Table.** Recommended seeding densities and working volume

## PERFORMANCE

### Isolation of hMSCs from Umbilical Cord

#### Day 0: Tissue Explantation

- Explant umbilical cord (UC) tissue in T-Flasks with the MAGNOLIA medium, containing Supplement Mix and 2 % Human AB Serum.
- Incubate to allow cell spreading. Observe round-shaped cells migrating from the tissue.

#### Day 3: Medium Refresh

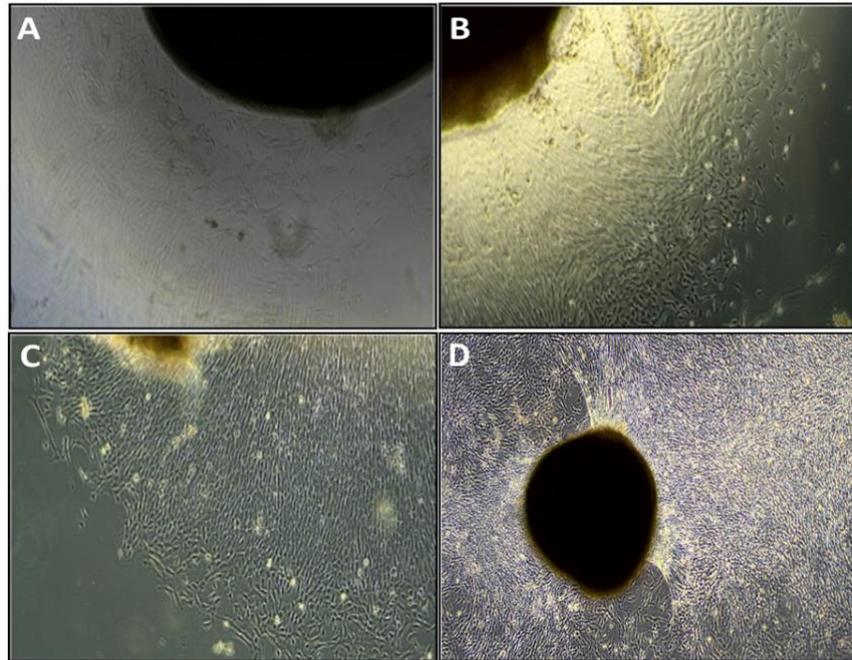
- Refresh the culture with pre-warmed MAGNOLIA medium containing Supplement Mix and 2 % Human AB Serum to provide new nutrients and to remove waste.

#### Days 6, 9, 12, 15: Cell Attachment and Proliferation

- At this stage, secreted cells from the tissue begin to attach to the flask surface and show signs of proliferation.
- Every 3 days, refresh the medium to provide fresh nutrients and to remove metabolic waste.

#### Day 18: Cell Dissociation

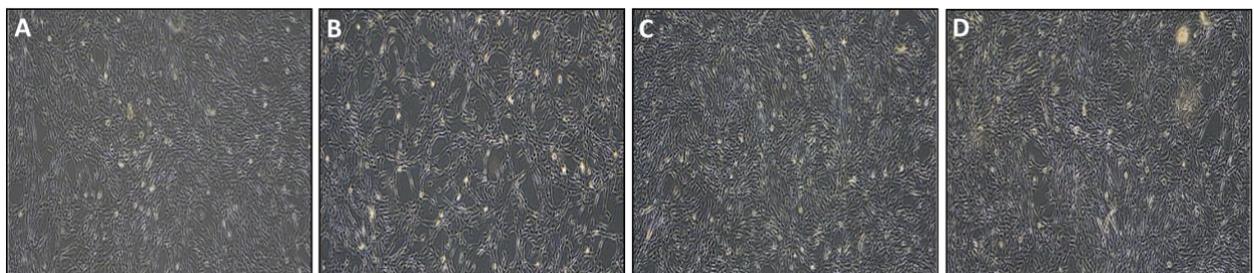
- After 18 days, the tissue pieces will have shrunk. Carefully remove them from the flasks.
- Dissociate (trypsin treatment) the attached cells from the flask surface. Thus, isolate the attached cells and define the passage 0 of the isolated hMSC (figure 1).



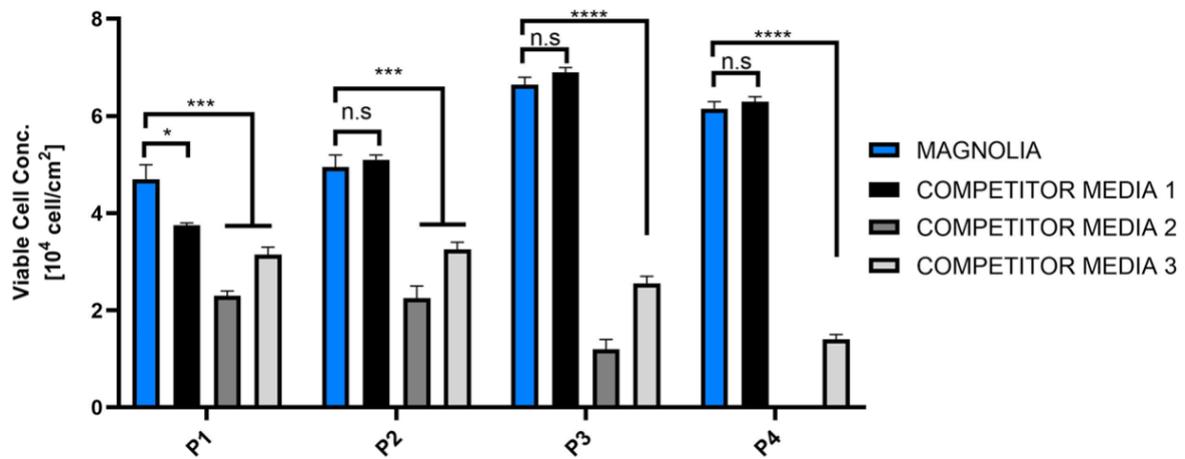
**Figure 1.** hMSC isolation from umbilical cord in MAGNOLIA medium, supplemented with Supplement Mix and 2 % Human AB Serum. Representative images are taken A) day 6, B) day 10, C) day 14, and D) day 18 after tissue explants.

## Proliferation of hMSCs

Human mesenchymal stem cells isolated from umbilical cord (hMSCs) were cultured over four passages. An inoculation cell concentration of 5000 cells/cm<sup>2</sup> (appr. 4 x 10<sup>5</sup> cells in T75 flask) was used. Experiment was performed in T75 flasks. Seeding cell concentration was kept constant through out all splits. Cells were passaged every 3<sup>rd</sup> or 4<sup>th</sup> day after inoculation depending on donor. Microscopic images were captured at each passage to monitor cell morphology and growth (figure 2). An OLS CASY automatic cell counter was employed to determine cell concentration (figure 3). Average cell number was 3 – 5 x 10<sup>6</sup> cells in one T75 flask after 3- or 4-days cultivation. This represents a cell amplification of 10 times in one passage.



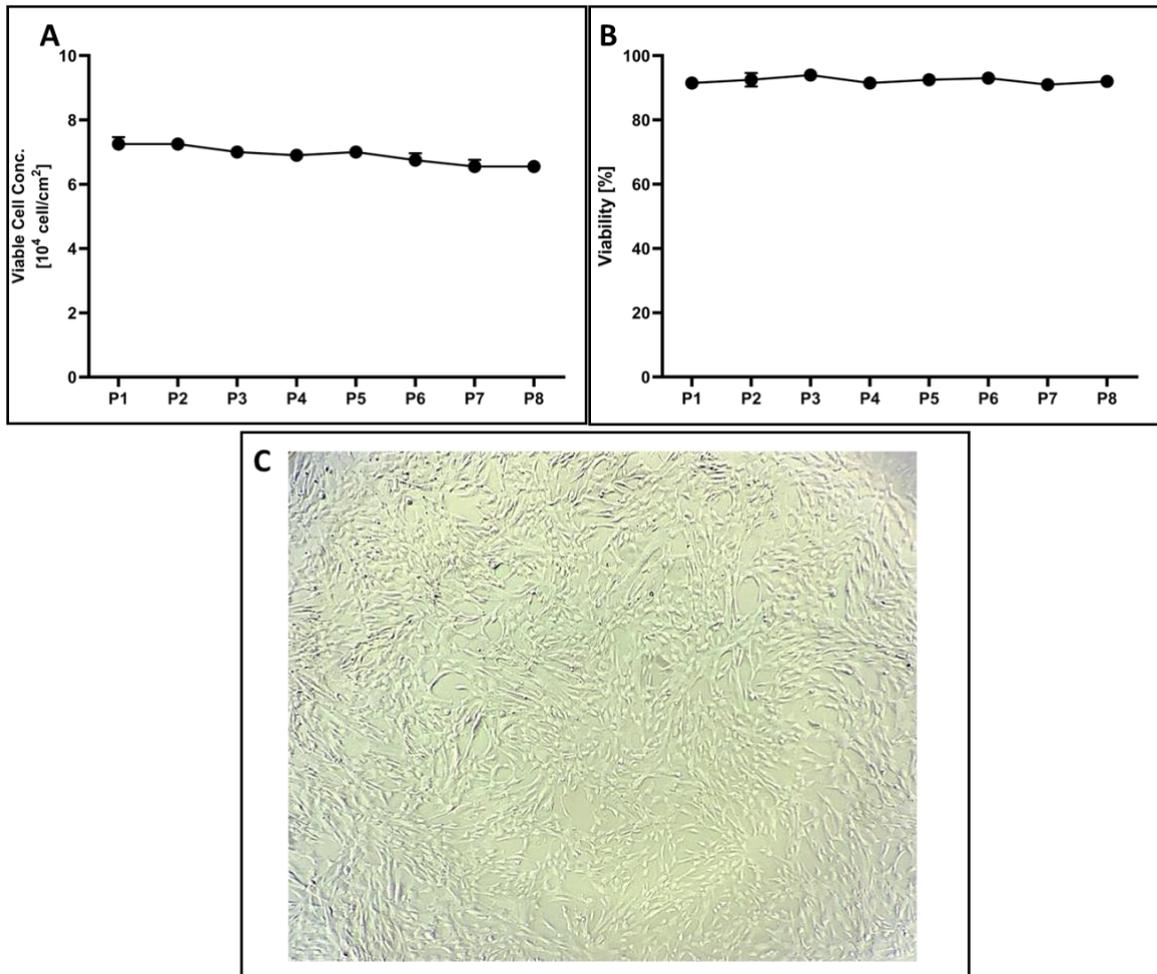
**Figure 2.** Expansion of hMSC from umbilical cord in MAGNOLIA medium A) passage 1, B) passage 2, C) passage 3, and D) passage 4. Seeding cell concentration was 5000 cells/cm<sup>2</sup> in each passage. Images were taken at day 3 post seeding.



**Figure 3.** hMSC proliferation for 4 passages in MAGNOLIA medium and competitor's media. Cell proliferation was assessed by cell count using OLS CASY cell counter. Data represented the mean  $\pm$  SEM. Two-way ANOVA statistical analysis was used (n=2) (\*p<0.05, \*\*p<0.01, \*\*\*p<0.005, \*\*\*\*p<0.001).

### Long term cultivation of hMSCs

Human mesenchymal stem cells (hMSCs) were isolated from umbilical cord and cultured over eight passages. An inoculation cell concentration of 5000 cells/cm<sup>2</sup> (appr.  $4 \times 10^5$  cells in T75 flask) was used and experiment was performed in treated T75 flasks. Seeding cell concentration was kept constant through out of all splits. Cells were passaged by a confluency between 80-95 % which is normally achieved by every 3<sup>rd</sup> or 4<sup>th</sup> days after inoculation depending on donor. Viable cell concentration and viability were measured in every passage (OLS CASY automatic cell counter) and microscopy images were captured at the end of passage 8 to monitor cell morphology (figure 4). Average cell number was 3 – 5 x 10<sup>6</sup> cells in one treated T75 flask after 3 or 4 days cultivation. This represents a cell amplification of 10 times in one passage.



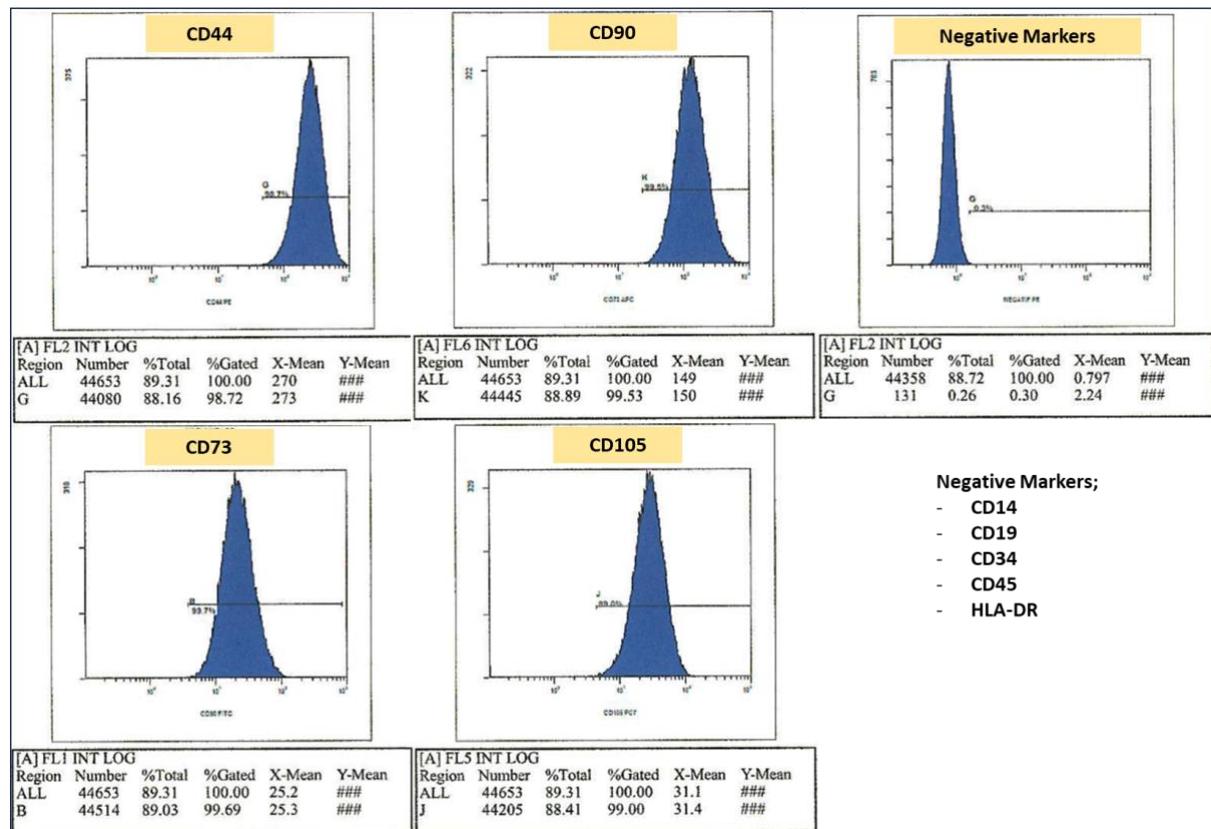
**Figure 4.** hMSC proliferation for 8 passages in MAGNOLIA medium, supplemented with Supplement Mix and 2 % Human AB Serum. Cell proliferation was assessed by cell counting using OLS CASY cell counter A) Viable cell concentration, B) Viability and C) Microscopic image at the end of passage 8. Data represented the mean  $\pm$  SEM.

## Characterization of hMSCs

hMSCs were isolated from approximately 20 different donors, cultured in Magnolia medium and cells were characterized as below. The cell characteristic data were always the same, representing the donor independent functionality and general applicability of medium.

At the end of 4<sup>th</sup> cell passage, cells were characterized using flow cytometry (Beckman Coulter, CytoFlex). For immunophenotyping, specific positive markers (CD44, CD73, CD90, CD105) were identified, with positivity exceeding 95 % in cell population, confirming the stem cell identity of the hMSCs after 4 passages. In contrast, negative markers (CD14, CD19, CD34, CD45, HLA-DR) were present at levels lower than 0.5 %, indicating the absence of non-stem cell lineages after 4 passage sub-cultivation of hMSCs (figure 5). The results demonstrate that the hMSCs maintained their stem cell characteristics across passages, suggesting that Magnolia medium offers an

effective nutritional environment for their sustained growth and cell characteristics. This underscores the potential of MAGNOLIA medium in stem cell research and applications.



**Figure 5.** Immunophenotyping results of hMSC after 4 passages using FACS analysis. As positive markers CD44 (98.72%), CD90 (99.53%), CD73 (99.69%), CD105 (99%) and negative markers (0.3 % of CD14, CD19, CD34, CD45, HLA-DR) were analyzed.

## MEDIA SUPPLY SECURITY

|   |  |
|---|--|
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|  |  |

- Our media are manufactured by partners, Eminence Scientific (China) and Capricorn Scientific (Germany), ensuring a consistent global supply from two strategic locations.
- Produced under GMP standards by Eminence Scientific or Capricorn Scientific, our media comply with EMA and FDA regulations.
- Available in liquid form, in GMP or non-GMP grades, tailored to customer specifications.
- GMP batch sizes reach approximately 2,000 L of liquid media.
- Media are supplied directly by Eminence Scientific or Capricorn Scientific.
- Through our extensive distribution network, we deliver to nearly every country worldwide.
- We invite our valued customers to tour our cutting-edge production facilities in Germany or China.

## TECHNICAL SUPPORT

| Developer   | Contact              |
|---|----------------------|
|  | info@florabio.com.tr |

The developer of the culture media and solutions is Florabio A.S. We welcome our valued customers to contact us about any questions you might have in media characteristics or application of medium in your lab. We would be please to share our experience with you.

## ORDERING INFORMATION

| <b>Name</b>  | <b>Application</b>  | <b>Formulation</b> | <b>Volume</b> | <b>Catalogue number</b> |
|--|---------------------|--------------------|---------------|-------------------------|
| <b>MAGNOLIA</b>                                      | Culture medium      | Liquid             | 0.5 L         | MNL1-L05                |
| <b>MAGNOLIA<br/>w/o phenol red</b>                   | Culture medium      | Liquid             | 0.5 L         | MNL2-L05                |
| <b>MAGNOLIA<br/>Supplement Mix</b>                   | Supplement Mix      | Liquid             | 1 mL          | SPL1-L0001              |
| <b>DPBS w/o Ca&amp;Mg</b>                            | Buffer Solution     | Liquid             | 1 L           | DPS2-L1                 |
| <b>0.25% Trypsin-EDTA<br/>Solution w/o Ca&amp;Mg</b> | Dissociation Reagen | Liquid             | 0.1 L         | TEA1-L01                |

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