

## Chondrocyte Differentiation for Umbilical Cord Derived MSCs

Catalog No.	Product Name	Product quantity	Short-term Storage	Long-term Storage	Thawing Instructions
ax9003	MSCs (Umbilical Cord Derived)	500,000 cells/vial	Liquid Nitrogen	Liquid Nitrogen	See Protocol
ax9005	MSC Expansion Medium for Adipose Tissue Derived & Umbilical Cord Derived MSCs	500 mL	4°C for 1 month	-20°C for 6 months	Thaw at 4°C or RT
ax9009	MSC Chondrogenesis Medium	100 mL	Store at 4°C for up to 6 weeks	N/A	N/A

### Recommendations:

- Recommended culture vessel coating: Not required
- Recommended cell culture medium: Axol MSC Expansion Medium followed by Axol MSC Chondrogenesis Medium (see Table)
- Recommended centrifugation speed: 200 x g for 5 min

### Seeding:

- Expand the **MSCs (Umbilical Cord Derived)** in **MSC Expansion Medium for Adipose Tissue Derived & Umbilical Cord Derived MSCs** until the cells are actively proliferating.
- Passage when the culture reaches: 70-90% confluent
- Recommended passaging reagent: Trypsin-EDTA
- Neutralize the trypsin with pre-warmed cell culture medium and centrifuge the cells at 200 x g for 5 min.
- Remove the supernatant and resuspend in 1-2 mL of pre-warmed cell culture medium.
- Perform a cell count to determine the number of viable cells.
- Resuspend the cells in **MSC Expansion Medium** at a concentration of **1.6 x 10<sup>7</sup> cells/mL**.
- Seed 5 µL drops of cells into multi-well plates (no more than 1 drop per 0.33 cm<sup>2</sup>).
- Incubate the plates for 2 h at 37°C, 5% CO<sub>2</sub> in a humidified incubator. Ensure that the incubator is adequately humidified.
- Gently add pre-warmed **MSC Chondrogenesis Medium** to the wells (2 mL per well for a 6-well plate).

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## Differentiation (21 Days):

- The cells should be maintained at 37°C, 5% CO<sub>2</sub> in a humidified incubator.
- Gently replace the culture medium every 2-3 days with fresh, pre-warmed **MSC Chondrogenesis Medium**.
- At Day 21 after seeding, differentiation will be complete. Fix the cells with 4% paraformaldehyde and stain for sulfated proteoglycans such as by using Alcian Blue stain.

## Usage Statement:

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans.

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