Osteocyte 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
ax9010	MSC Osteogenesis Medium	100 mL	Store at 4°C for up to 6 weeks	N/A	N/A

Recommendations:

- Recommended culture vessel coating: Fibronectin
- Recommended cell culture medium: Axol MSC Expansion Medium followed by Axol MSC Osteogenesis Medium (see Table)
- Seeding density for Osteogenesis: 15,000-20,000 viable cells/cm^2
- Recommended centrifugation speed: 200 x *g* for 5 min

Coating:

- Coat the required number of 6-well plates with Fibronectin Coating Solution.
- Dilute the stock **Fibronectin Coating Solution 1:50** in sterile water to make 1x working solution e.g. **100 µL** in **5 mL**.
- On the day prior to thawing the cells, coat the surface of your culture vessel with the **Fibronectin** 1x working solution. We recommend coating at a volume of **200 µL per cm^2** however, please optimize for your experiments.
- Incubate the culture vessel **overnight** at **37°C** in a humidified incubator.
- Remove the coating solution prior to seeding the cells.

Coating the culture vessels with Fibronectin will prevent the cells from detaching during differentiation

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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.
- Seed cells into the 6-well plate (coated with Fibronectin) at the recommended seeding density (15,000-20,000 viable cells/cm^2) in pre-warmed **MSC Expansion Medium** (2 mL per well for a 6-well plate).
- Incubate the cells at 37°C, 5% CO_2 in a humidified incubator.

Differentiation (21 Days):

- The cells should be maintained at 37°C, 5% CO_2 in a humidified incubator.
- At 24 h post-seeding, gently remove the **MSC Expansion Medium** and rinse the wells with 1x PBS once.
- Remove the PBS and add pre-warmed **MSC Osteogenesis Medium** (2 mL per well for a 6-well plate).
- Gently replace the **MSC Osteogenesis Medium** every 3-4 days, following the notes below.
- Gently remove 75% of the spent cell culture medium and gently dispense an equivalent volume of fresh, pre-warmed culture medium down the side of the well. Do not tilt the plate when removing the culture medium and do not remove all of the culture medium.

The monolayer is fragile and must be handled with extreme care or it may detach from the plate

- At Day 21 after seeding, differentiation will be complete. Fix the cells with 4% paraformaldehyde and stain for calcium deposition such as by using Alizarin Red 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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