Adipocyte 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
ax9019	MSC Adipogenesis Medium for Bone Marrow Derived and Umbilical Cord Derived MSCs	100 mL	Store at 4°C for up to 6 weeks	N/A	N/A
ax0049	Fibronectin Coating Solution	1 mL	Aliquot and store at -80°C for up to 3 months	Thaw at 4°C	Once diluted, use immediately

Recommendations:

- Recommended culture vessel coating: Fibronectin
- Recommended cell culture medium: Axol MSC Expansion Medium followed by Axol MSC Adipogenesis Medium (see Table)
- Seeding density for Adipogenesis: 40,000-60,000 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

Address: Axol Bioscience Limited, Suite 3, The Science Village, Chesterford Research Park, Little Chesterford, Cambridgeshire, CB10 1XL
Phone: +44 (0) 1223 751 051 | US Toll Free Tel: 1-800-678-2965
Email: support@axolbio.com
Web: www.axolbio.com

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 (40,000-60,000 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 (28 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 Adipogenesis Medium** (2 mL per well for a 6-well plate).
- Replace the **MSC Adipogenesis Medium** every 3-4 days. Gently remove the spent cell culture medium and dispense pre-warmed fresh culture medium down the side wall of the well.
- At Day 28 after seeding, differentiation will be complete. Fix the cells with 4% paraformaldehyde and stain for lipid accumulation such as by using Oil Red O 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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