

# **Protocol version 1.0**

### **Human Mesenchymal Stem Cells (Umbilical Cord Derived)**

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 below
ax9005	MSC Expansion Medium for Umbilical Cord- Derived MSCs	500 mL	4°C for 1 month	-20°C for 6 months	Thaw at 4°C or RT

Lot-specific information such as donor details and passage number are stated in the Certificate of Analysis for each product.

#### **Recommendations:**

#### Always count the number of viable cells after thawing

Recommended culture vessel coating: Not required

• Recommended cell culture medium: Axol MSC Expansion Medium for Umbilical Cord-

**Derived MSCs** 

• Recommended seeding density: 5,000-10,000 viable cells/cm<sup>2</sup>

• Recommended centrifugation speed: 200 x g for 5 min

### **Thawing & Plating:**

- Thaw the cells quickly in a 37°C water bath until just prior to complete thawing
- Wipe the outside of the vial with 70% ethanol
- Gently resuspend the cells and take an aliquot to perform a cell count.
- Slowly dilute the cells into the required volume of pre-warmed **MSC Expansion Medium** (must be at least 10 mL so that the concentration of DMSO is less than 1%).
- Rinse the cryovial with 1 mL of MSC Expansion Medium to ensure all of the cells are transferred.
- Seed cells into the culture vessel at the recommended seeding density
- Once the cells have attached (after 6-24 h), replace the culture medium with fresh prewarmed MSC Expansion Medium

Frequency of media changes: Every 2-3 days

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#### **Passaging:**

Passage when the culture reaches: 70-90% confluent
 Recommended passaging reagent: Trypsin/EDTA

- Neutralize the trypsin with MSC Expansion Medium and centrifuge the cells at 200 x g for 5 min
- Remove the supernatant and resuspend in 1-2 mL of pre-warmed MSC Expansion
   Medium
- Perform a cell count to determine the number of viable cells
- Dilute the cells into the required volume of pre-warmed MSC Expansion Medium
- Seed cells into the culture vessel at the recommended seeding density

It is recommended that the Mesenchymal Stem Cells are used for endpoint assays prior to passage 6 for optimal performance in your experiments

#### **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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