# Human Dental Pulp Stem Cells

| Catalog No. | Product Name | Product Quantity | Storage on Arrival | Thawing Instructions | Storage Once Thawed |
|---|---|---|---|---|---|
| ax3901 | Dental Pulp Stem Cells | 1 million cells/vial | Liquid nitrogen | Follow protocol | N/A |
| ax3902 | Dental Pulp Stem Cell Culture Medium | 500 mL | Aliquot and store at -20°C for up to 6 months | Thaw at 4°C or room temperature | Store at 4°C for up to 1 month |
| ax0044 | Unlock | 25 mL | Aliquot & store at -80°C for up to 6 months | Thaw at 4°C | Store at 4°C for up to 1 week |
| ax9009 | MSC Chondrogenesis Medium | 100 mL | Aliquot and store at -20°C for up to 6 months | Thaw at 4°C or room temperature | Store at 4°C for up to 1 month |
| ax9010 | MSC Osteogenesis Medium | 100 mL | Aliquot and store at -20°C for up to 6 months | Thaw at 4°C or room temperature | Store at 4°C for up to 1 month |
| ax9019 | MSC Adipogenesis Medium for Bone Marrow Derived and Umbilical Cord Derived MSCs | 100 mL | Aliquot and store at -20°C for up to 6 months | Thaw at 4°C or room temperature | Store at 4°C for up to 1 month |
| ax0049 | Fibronectin Coating Solution | 1 mL | Aliquot and store at -80°C for up to 3 months | Thaw at 4°C | Once diltued, use immediately |

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

# General Recommendations:

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

- Recommended culture vessel coating: Not required
- Recommended cell culture medium: **Dental Pulp Stem Cell Culture Medium**
- Recommended seeding density: **5,000 viable cells/cm$^2$**
- Recommended centrifugation speed: **200 x *g* for 5 minutes**

**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

## Thawing & Plating:

- Transfer the vial of cells from liquid nitrogen storage with the vial buried in dry ice. Remove the vial from dry ice and transfer it immediately to a **37°C** water bath.
- 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 transfer to a 15 mL sterile conical tube.
- Slowly add **10 mL** of pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium**.
- Rinse the cryovial with 1 mL of **Dental Pulp Stem Cell Culture Medium** to ensure all of the cells are transferred.
- Centrifuge the cells at **200 x *g*** for **5 minutes**.
- Carefully remove the supernatant and resuspend the cell pellet in **1-2 mL** of pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium**.
- Seed cells into the culture vessel at the recommended seeding density of **5,000 viable cells/cm$^2$**.
- Incubate the cells at **37°C**, **5% $CO_2$** in a humidified incubator.
- After **24 h**, replace the culture medium with fresh pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium**.
- Replace the culture medium **every 1-2 days** depending on confluency.

## Passaging:

- Passage when the culture reaches: **80-100% confluent**
- Recommended passaging reagent: **Unlock**

- Remove all spent medium from cell culture vessels.
- Gently rinse the surface of the cell layer once with PBS, 2 mL of PBS per 10 cm$^2$ culture surface area. Discard the PBS.
- Add 1 mL per 10 cm$^2$ of culture surface area of **cold/room temperature Unlock** passaging reagent. Evenly distribute it over the entire cell layer.
- Incubate the cells for **5 minutes** at **37°C**. Observe the cells at regular intervals for detachment from the culture vessel.
- Once the cells have detached, dilute out the passaging reagent with four volumes pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium**. For example, if 1 mL of **Unlock** is used, then add 4 mL of the medium to stop the reaction.
- Transfer the cell suspension to a sterile conical tube.
- Centrifuge the cells at **200 x *g*** for **5 minutes**.

**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

- Carefully remove the supernatant and resuspend the cell pellet in **1-2 mL** of pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium**.
- Seed cells into the culture vessel at the recommended seeding density of **5,000 viable cells/cm$^2$** or as appropriate for subsequent lineage differentiation (see below).
- Incubate the cells at **37°C**, **5% $CO_2$** in a humidified incubator.

**It is recommended that the Dental Pulp Stem Cells are not passaged more than 3 times since early passage cells perform better for subsequent lineage 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

# Differentiation to Adipocytes:

## Recommendations:

- Recommended culture vessel coating: **Fibronectin**
- Recommended cell culture medium: **MSC Adipogenesis Medium (ax9019)**
- Recommended seeding density: **40,000-60,000 viable cells/cm$^2$**

## Coating:

- Dilute the stock **Fibronectin Coating Solution 1:100** in sterile water to make 1x working solution e.g. **100 µL** in **10 mL**.
- Coat the required number of 6-well plates 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.

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

## Adipocyte Differentiation (28 days):

- Seed cells into the **fibronectin-coated** culture vessel at the recommended seeding density of **40,000-60,000 viable cells/cm$^2$** in pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium**.
- The cells should be maintained at **37°C**, **5% $CO_2$** in a humidified incubator.
- At **24 h** post-seeding, gently remove the **Dental Pulp Stem Cell Culture Medium** and rinse the wells with PBS once.
- Remove the PBS and add pre-warmed, **37°C**, **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, **37°C**, 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.

**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

# Differentiation to Chondrocytes:

## Recommendations:

- Recommended culture vessel coating: Not required
- Recommended cell culture medium: **MSC Chondrogenesis Medium (ax9009)**
- Recommended seeding density: **1.6 x $10^7$ cells/mL**

## Chondrocyte Differentiation (21 days):

- Resuspend the cells in pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium** at a concentration of **1.6 x $10^7$ cells/mL**.
- Seed **5 µL** drops of cells into multi-well plates (no more than 1 drop per 0.33 $cm^2$).
- Incubate the plates for **2 h** at **37°C**, **5% $CO_2$** in a humidified incubator. Ensure that the incubator is adequately humidified.
- Gently add pre-warmed, **37°C**, **MSC Chondrogenesis Medium** to the wells (2 mL per well for a 6-well plate).
- The cells should be maintained at **37°C**, **5% $CO_2$** in a humidified incubator.
- Gently replace the culture medium every 2-3 days with pre-warmed, **37°C**, fresh **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.

**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

# Differentiation to Osteocytes:

## Recommendations:

- Recommended culture vessel coating: **Fibronectin**
- Recommended cell culture medium: **MSC Osteogenesis Medium (ax9010)**
- Recommended seeding density: **15,000-20,000 viable cells/cm$^2$**

## Coating:

- Dilute the stock **Fibronectin Coating Solution 1:100** in sterile water to make 1x working solution e.g. **100 µL** in **10 mL**.
- Coat the required number of 6-well plates 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.

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

## Osteocyte Differentiation (21 days):

- Seed cells into the **fibronectin-coated** 6-well plate at the recommended seeding density of **15,000-20,000 viable cells/cm$^2$** in pre-warmed, **37°C**, **Dental Pulp Stem Cell Culture Medium** (2 mL per well for a 6-well plate).
- The cells should be maintained at **37°C**, **5% $CO_2$** in a humidified incubator.
- At **24 h** post-seeding, gently remove the **Dental Pulp Stem Cell Culture Medium** and rinse the wells with PBS once.
- Remove the PBS and add pre-warmed, **37°C**, **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, **37°C**, 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.

**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

## 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.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
International phone **+44-1223-751-051**
US phone **+1-800-678-AXOL (2965)**

**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