

AXOL
Discovery Stems From Here

The logo features the word 'AXOL' in a bold, white, sans-serif font. The letter 'O' is replaced by a circular arrangement of small, multi-colored dots (blue, green, yellow, red) that form a ring. Below the logo, the tagline 'Discovery Stems From Here' is written in a smaller, white, sans-serif font. The background is a dark blue gradient with a dense field of small, glowing particles and larger, semi-transparent blue circles of varying sizes, creating a sense of depth and movement.

3D Cell Culture – Matrix & Solution

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax134445	3D Cell Culture Matrix	1 x 1mL	N/A	Most stable when stored at +4°C for 6 months	Follow protocol	It is not recommended to aliquot and re-freeze samples of unused product
ax134446	3D Cell Culture Matrix	5 x 1mL	N/A	Most stable when stored at +4°C for 6 months	Follow protocol	It is not recommended to aliquot and re-freeze samples of unused product
ax134447	3D Cell Culture Solution	25 mL	N/A	Most stable when stored frozen at -20°C (or at -80°C for longer-term storage) until used	Follow protocol	Upon thawing, it is recommended to aliquot and re-freeze samples of unused Solution at -20°C. For use, pre-warm only an aliquot and keep the remaining medium refrigerated at 4 to 8°C
ax134448	3D Cell Culture Solution	100 mL	N/A	Most stable when stored frozen at -20°C (or at -80°C for longer-term storage) until used	Follow protocol	Upon thawing, it is recommended to aliquot and re-freeze samples of unused Solution at -20°C. For use, pre-warm only an aliquot and keep the remaining medium refrigerated at 4 to 8°C
ax134449	3D Matrix Accelerator	50 µL	N/A	Store at -20°C	N/A	N/A
ax1344412	3D Matrix Accelerator	250 µL	N/A	Store at -20°C	N/A	N/A
ax1344411	3D Cell Culture Supplement-XF	500 µL	N/A	Store at +4°C	N/A	N/A
ax1344415	3D Matrix kit: Ax134445 3D Cell Culture Matrix Ax134449 3D Matrix Accelerator	Kit	N/A	Follow the single product information	Follow the single product information	Follow the single product information
ax1344416	3D Matrix kit: Ax134446 3D Cell Culture Matrix Ax1344412 3D Matrix Accelerator	Kit	N/A	Follow the single product information	Follow the single product information	Follow the single product information
ax1344413	3D Solution kit: Ax134447 3D Cell Culture Solution Ax1344411 3D Cell Culture Supplement-XF	Kit	N/A	Follow the single product information	Follow the single product information	Follow the single product information
ax1344414	3D Solution kit: Ax134448 3D Cell Culture Solution Ax1344411 3D Cell Culture Supplement-XF	Kit	N/A	Follow the single product information	Follow the single product information	Follow the single product information
ax134443	3D Cell Culture Kit Multi Use: Ax1344416 3D Matrix kit Ax1344414 3D Solution kit	Kit	N/A	Follow the single product information	Follow the single product information	Follow the single product information
ax134443	3D Cell Culture Kit Single Use: Ax1344415 3D Matrix kit Ax1344413 3D Solution kit	Kit	N/A	Follow the single product information	Follow the single product information	Follow the single product information

3D Cell Culture Kit

Three-Dimensional (3D) cell culture is an artificially created environment in which cells are permitted to grow or interact with their surroundings. The 3D environment improves the function, differentiation and viability of cells and recapitulate *in vivo* microenvironment compared to conventional 2D cell culture experiments.

Axol 's 3D cell culture kit include:

- Lyophilized Cell Culture Matrix derived from human platelet rich plasma that allow cells to grow in several layers at the interface layer which minimize contact inhibition with any artificial biomaterials.
- Matrix Accelerator contains human coagulation factors, that enhances the gelatinization process of the matrix.
- 3D Cell Solution, a high-performance cell culture supplement derived from human platelet units containing abundant growth factors and cytokines.
- Solution Supplement-XF to avoid coagulation.

Important Note

Axol's 3D cell culture Matrix and Solution supports the *in vitro* propagation and maintenance of various human cell types, including Dermal Fibroblasts, Epidermal Keratinocytes and Endothelial Colony Forming Cells. The 3D Cell Culture Matrix and Solution has been used and developed extensively using Mesenchymal Stem Cells (MSC), the preparation of the reagents and the protocols are developed in regard to the MSCs. The 3D products are being continuously tested on different cell types for their properties and efficiency. Use this Protocol as a reference tool when using with other cells type.

Preparation of the Reagent

3D Cell Culture Solution

- Upon receipt, store 3D Cell Culture Solution at -20°C (or at -80°C for longer-term storage) until used. Once thawed, aliquot and re-freeze samples of unused Solution at -20°C.
- When ready to use, pre-warm only an aliquot of 3D Cell Culture Solution for 1 hour in a 37°C water bath or overnight at 4°C and keep the remaining medium at 4°C.
- Prepare a complete cell culture media by adding the 3D Cell Culture Solution to basal medium (i.e. Dulbecco's Modified Eagles Medium-Low Glucose; DMEM-LG) with a final concentration of 2 mM L-glutamine and 100 U/mL penicillin-streptomycin. 3D Cell Culture Solution shows optimal growth of Mesenchymal Stem Cells (MSC) at 5% (v/v). However, for higher cell proliferation rates, it is recommended to use 10% (v/v) of the solution. Complete medium can be stored at 4°C and is stable for approximately four weeks.
- To avoid coagulation of the medium, heparin need to be added at a final concentration of 0.024 mg/mL, use Axol 3D Supplement-XF for this purpose.

3D Cell Culture Matrix

- Upon receipt, store 3D Cell Culture Matrix at 4°C for 6 months. It is not recommended to aliquot and re-freeze samples of unused product.
- When ready to use, reconstitute one vial of Cell Culture Matrix in 1 mL double-distilled water (ddH₂O).
- Prepare Complete 3D Cell Culture Matrix by adding reconstituted matrix to basal medium (i.e. Dulbecco's Modified Eagles Medium-Low Glucose; DMEM-LG) with 2 mM L-glutamine and 100 U/mL penicillin-streptomycin as final concentration and mix gently (Follow the protocol). The matrix shows optimal gelatinization at 10% (v/v).

Culture of Human Mesenchymal Stem Cells

Recommendations

- Recommended cell culture medium: Axol MSC Expansion Medium.
- Recommended seeding density: 5,000-10,000 viable cells/cm².
- 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 days.

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.

Protocol Overview

General protocol for embedding cells in three-dimensional environment

- On the day of the experiment use the MSCs previously thawed, plated and ready to use.
- Passage the cells using Trypsin/EDTA, 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 trypsin with complete MSC Expansion Medium and centrifuge the cells at 200 x g for 5 min.
- Remove the supernatant.

NB. (if the starting point of the cells is different for the one presented here {i.e. frozen cells, cultured cells, still in the tissue culture flask or collected in a tube, etc.} just resuspend the cells, regardless the condition, with basal medium and then add the reconstitute 3D Matrix).

- Resuspend the cells with basal medium containing calcium (i.e. Dulbecco's Modified Eagles Medium-Low Glucose; DMEM-LG), and a final concentration of 2 mM L-glutamine and 100 U/mL penicillin-streptomycin. Plate the cells in a Flask.
- Reconstitute one vial of Complete 3D Cell Culture Matrix in 1 mL ddH₂O.
- Transfer the reconstitute 3D Cell Culture Matrix to the cell culture plated in the flask and mix gently by rotation of the flask.
- Allow gelatinization of the 3D Cell Culture Matrix by incubating tissue culture flasks for 60min at 37°C. Note: Do not disturb the gelation process during that time.
- To enhance the gelation process, add 50 µg/mL of 3D Accelerator to the complete 3D cell culture mixture to allow gelatinization by incubating the culture plate at 5% CO₂, 37°C for 2min.
- After gelatinization, overlay the 3D cell culture mixture with 3D Cell Culture Solution medium and incubate the flask at 5% CO₂ and 37°C.
- When passaging, cells can be harvested together with the gel by pipetting and then re-plating onto cell culture flasks prepared with fresh Cell Culture Matrix.
- Exchange 3D Cell Culture Solution 48 hours after seeding and then every third day. Only remove the 3D Cell Culture Solution, not matrix, and replace it with a fresh Solution.

General protocol for single-cell analysis

NB. When collecting the cells which are embedded in the 3D Cell Culture Matrix, you need to remove first the 3D Cell Culture Solution and collect only the gel (which contains the cells).

- Discard the 3D Cell Culture Solution from the cell plate, leaving matrix untouched.
- Collect the 3D Cell Culture Matrix gel (with the cells) and residual of the Solution to a sterile 15 mL conical tube.
- Centrifuge cells at 350 x g for 5min.
- Carefully remove and discard the supernatant (be careful not to disrupt the gel pellet).
- Resuspend the cell pellet and gel pellet with 1 mL Trypsin/EDTA (0.25%) by gently pipetting 5 to 10 times, until the cells are in a single cell suspension.
- Add 9 mL of 3D Cell Culture Solution Culture Medium to the cell suspension.
- Centrifuge cells at 350 x g for 5min at room temperature.
- Remove the supernatant and resuspend the cell pellet in 1 mL 3D Cell Culture Solution medium, perform the cell counting.

Recommendation

For ideal cell expansion from cell culture flasks, it is recommended to use tissue culture flask with peel-off foil in order to provide a total access from above without any hindrance.

Once the lyophilized matrix pellet is reconstituted in ddH₂O and after very well mixing you should notice a yellowish, slightly cloudy liquid. Mix the reconstituted matrix with basal medium in the desired concentration.

Best results have been observed with 10% (v/v). You can mix by pipetting up and down several times (avoid excessive foam formation).

Pipette the desired amount into a cell culture plate and place the plate in the incubator (37°C) without shaking.

Gel formation takes about 60 minutes. Do not disturb the gelation process during that time. If the gelation process is disturbed by strong shaking/movements the gel will NOT form anymore.

After 60 minutes check if a proper gel has formed. The entire volume should have turned into a very soft but stable gel. The gel should look like a liquid (smooth surface) but is solid enough to keep its shape when you hold the plate upside-down. If the gel has a slightly wrinkled surface, wait a few more minutes.

To induce the gelation process, it is recommended to add 50 µg/mL of 3D Accelerator to the complete 3D cell culture mixture to allow gelatinization after 2 minutes incubation at 37°C.

Particulate formation when using 3D Cell Culture Solution does not affect cell culture performance. If clotting or insoluble particles appears in final medium, it is recommended to filter the complete MSC culture medium after diluting in the basal medium to remove insoluble particles.

Avoiding multiple freeze/thaw cycles of thawed 3D Cell Culture Solution can minimize particulate formation.

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 applications to humans.

Notes

Got any questions? Need help with the protocol?
Contact Axol Technical Support at
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Or
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