AXOL

Discovery Stems From Here

Human Fibroblasts

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax3010 ax3011 ax3012 ax3013 ax3014 ax3015 ax3016 ax3017 ax3018 ax3019 ax3020 ax3021 ax3022 ax3023 ax3024 ax3025 ax3027 ax3030 ax3031 ax3032 ax3033 ax3034 ax3035 ax3036 ax3037 ax3038 ax3039 ax3040	Human Fibroblasts	500,000 cells/vial	N/A	Liquid Nitrogen	Follow Protocol	N/A
ax3045	Fibroblast Plating & Growth Medium plus Growth Factors	500 mL	N/A	-20°C for 6 months	Thaw at 37°C	4°C for 1 month

Recommendations

- Always count the number of viable cells after thawing.
- Recommended culture vessel coating: Not required.
- Recommended cell culture medium: Fibroblast Plating & Growth Medium.
- Recommended seeding density: 4,000 viable cells/cm^2 .
- Recommended centrifugation speed: 200 x g for 5 minutes.

Preparation of the Reagent Fibroblast

Plating & Growth Medium

Axol's Fibroblast Plating & Growth Medium has been optimized to maximise the recovery and viability of our cryopreserved human fibroblasts upon revival. The Medium contains no antimicrobials and no phenol red.

Fibroblast Plating & Growth Medium is offered in a kit format composed of 480mL basal medium and associated growth factors: rh FGF basic factor 0.5 mL; rh Insulin factor 0.5 mL; Ascorbic Acid 0.5 mL; L-Glutamine 18.75 mL; Hydrocortisone Hemisuccinate 0.5 mL; FBS 10 mL.

The kit allows you to prepare fresh complete medium in your laboratory, extending shelf life and enhancing performance.

Prior to using media with cells, always pre-warm the media for 10min at 37°C. Thaw frozen factors immediately prior to adding to the basal medium. L-Glutamine should be warmed to 37°C in a water bath and mixed to dissolve any precipitates before use. Precipitate may also form in Insulin when first thawed. Gently pipette insulin solution up and down to dissolve precipitated insulin.

All the Factors are sufficient to supplement one 480 mL bottle of Fibroblast Plating & Growth Medium and must be fully added to produce a final concentration of:
rh FGF basic factor 5ng/mL; rh Insulin factor 5 µg/mL; Ascorbic Acid 50 µg/mL;
L-Glutamine 7.5 mM; Hydrocortisone Hemisuccinate 1 µg/mL and FBS 2%.

Mix supplemented medium by gently pipetting up and down with a large volume pipette (50 mL) or gently invert the tightly closed 500 mL bottle. Do not shake or froth the medium.

Product	Volume	Final Concentrations in Supplemented Medium	Storage
Fibroblast Complete Kit	2-8°C when prepared		
Fibroblast Basal Medium	480 mL	2-8°C	
Fibroblast Factors Kit	-20°C		
rh FGF basic factor	0.5 mL	5 ng/mL	-20°C
rh Insulin factor	0.5 mL	5 µg/mL	-20°C
Ascorbic Acid factor	0.5 mL	50 µg/mL	-20°C
L-Glutamine factor	18.75 mL	7.5 mM	-20°C
Hydrocortisone Hemisuccinate factor	0.5 mL	1 µg/mL	-20°C
Fetal Bovine Serum	10 mL	2%	-20°C
Optional Antimicrobial Supplement: Gentamicin and Amphotericin B (not supplied)	0.5 mL	Gentamicin 30 µg/mL Amphotericin B 15 ng/mL	-20°C

Protocol Overview

Thawing and plating of Human Fibroblasts

- 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 take an aliquot to perform a cell count.
- Immediately after thawing, slowly dilute the cells into the required volume of pre-warmed complete Fibroblast Plating & Growth Medium (must be at least 10 mL so that the concentration of DMSO is less than 1%).
- Rinse the cryovial with 1 mL of complete Fibroblast Plating & Growth Medium to ensure all of the cells are transferred.
- Seed cells into the culture vessel at the recommended seeding density of 4,000 viable cells/cm^2.
- Incubate the cells at 37°C, 5% CO^2 in a humidified incubator.
- Once the cells have attached (after 6-24 hours), replace the culture medium with fresh pre-warmed complete Fibroblast Plating & Growth Medium.
- Frequency of media changes: Every 3 days.

Culture of Human Fibroblasts

- Passage when the culture reaches: 80-90% confluent.
- Recommended passaging reagent: Trypsin-EDTA.
- After adding passaging reagent, 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 Fibroblast Plating & Growth Medium and centrifuge the cells at 200 x g for 5 minutes.
- Remove the supernatant and resuspend in 1-2 mL of pre-warmed complete Fibroblast Plating & Growth Medium.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed complete Fibroblast Plating & Growth Medium.
- Seed cells into the culture vessel at the recommended seeding density of 4,000 viable cells/cm^2.
- Incubate the cells at 37°C, 5% CO^2 in a humidified incubator

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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