Human Fibroblast Cell Culture Medium (Animal Component-Free)

Catalog No.	Product Name	Product quantity	Short-term Storage	Long-term Storage	Thawing Instructions
ax3103-500	Human Fibroblast Cell Culture Medium (Animal Component-Free)	500 mL	4°C for 1 month	-20°C or below for 6 months	Thaw at 4°C or RT
ax3037	Dermal Fibroblasts (Neonatal)	500,000 cells / vial	Liquid nitrogen	Liquid nitrogen	See below

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 Human Fibroblast Cell Culture Medium
- Recommended seeding density: 4,000 viable cells/cm^2
- Recommended centrifugation speed: 200 x *g* for 5 min

Thawing & Plating:

Axol Neonatal Human Dermal Fibroblasts (ax3037) can be thawed directly into **Axol Human Fibroblast Cell Culture Medium (Animal Component-Free; ax3103-500)**, following the instructions below:

- 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 cell culture medium (must be at least 10 mL so that the concentration of DMSO is less than 1%)
- Rinse the cryovial with 1 mL of 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
- Frequency of media changes: Every 3 days

Passaging:

- Passage when the culture reaches: 90% confluent
- Recommended passaging reagent: Trypsin/EDTA
- When the cells have detached from the culture vessel, dilute out the trypsin with cell culture medium and centrifuge the cells at 200 x *g* for 5 min

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It is important that the cells are centrifuged in order to remove the trypsin before plating the fibroblasts. Since the culture medium is serum-free, it does not contain trypsin inhibitors. Alternatively, a Xeno-Free trypsin inhibitor can be used.

- 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
- Dilute the cells into the required volume of pre-warmed cell culture medium
- Seed cells into the culture vessel at the recommended seeding density

Adapting Fibroblasts to Serum-Free Culture:

Human fibroblasts that have been cultured in serum-containing medium can be switched into our animal component-free and human derived component-free **Human Fibroblast Cell Culture Medium** by following the instructions below:

- Fibroblasts will tolerate the switch to serum-free medium better if they are actively proliferating and have high viability (>80%) before changing media
- Thaw the fibroblasts and seed into the serum-containing medium that they were cultured in prior to cryopreservation
- When the cells are actively proliferating, change the medium to a 50:50 mixture of the serum-containing medium and **Human Fibroblast Cell Culture Medium**
- When the fibroblasts reach 90% confluency, passage the cells into the 50:50 medium mixture
- When the fibroblasts are actively proliferating, change the medium to a 25:75 mixture of serum-containing medium (25%) and **Human Fibroblast Cell Culture Medium** (75%)
- When the fibroblasts reach 90% confluency, passage the cells into the 25:75 medium mixture
- When the fibroblasts are actively proliferating, change the medium to 100% **Human Fibroblast Cell Culture Medium**
- When the fibroblasts reach 90% confluency, passage the cells into 100% **Human Fibroblast Cell Culture Medium**
- It is recommended that you keep back-up flasks or vials during the adaptation process in case the fibroblasts require longer to adapt to serum-free conditions.
- An additional medium dilution step with a 10:90 ratio of serum-containing to serum-free medium may be required for cells that take longer to adapt.
- Although coating the culture vessels is not required, a Type I Collagen coating may increase cell attachment

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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