Assay-Ready Expanded Human Liver Sinusoidal Endothelial Cells

Catalog No.	Product Name	Product quantity	Short-term Storage	Long-term Storage	Thawing Instructions
ax3720	ARE Liver Sinusoidal Endothelial Cells	5 million cells/ vial	Liquid nitrogen	Liquid nitrogen	See below
ax3721	ARE Liver Sinusoidal Endothelial Cell Culture Medium	100 mL	4°C for 6 weeks	N/A	N/A

Lot-specific information such as donor information is stated in the Certificate of Analysis.

Recommendations:

- Always count the number of viable cells after thawing
- Recommended culture vessel coating: Type I Collagen
- Recommended cell culture medium: ARE Liver Sinusoidal Endothelial Cell Culture Medium
- Recommended seeding density: 5,000 viable cells/cm^2
- Recommended centrifugation speed: 620 x *g* for 5 min

Thawing & Plating:

Coat the culture vessels with Type I Collagen or use pre-coated culture vessels

- 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 **ARE Liver Sinusoidal Endothelial Cell Culture Medium**
- Rinse the cryovial with 1 mL of medium to ensure all of the cells are transferred
- Centrifuge the cells at 620 *x g* for 5 min
- Carefully remove the supernatant and resuspend in 2 mL of pre-warmed **ARE Liver Sinusoidal Endothelial Cell Culture Medium** and perform a cell count
- Dilute the cells into the required volume of pre-warmed **ARE Liver Sinusoidal Endothelial Cell Culture Medium**
- Seed cells into the culture vessel (coated with Type I Collagen) at the recommended seeding density
- Once the cells have attached (after 24 h), replace the culture medium with fresh pre-warmed **ARE Liver Sinusoidal Endothelial Cell Culture Medium**
- Frequency of media changes: Every 2 days with pre-warmed medium

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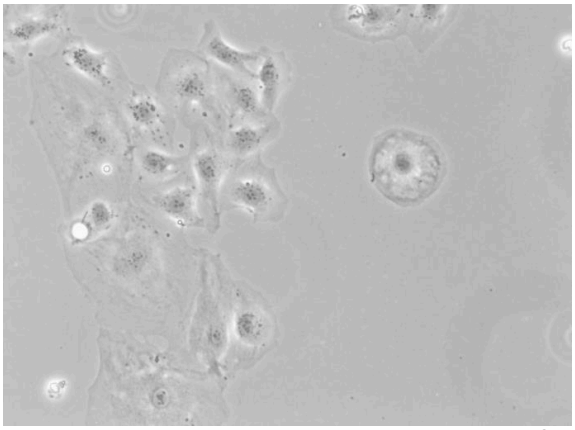
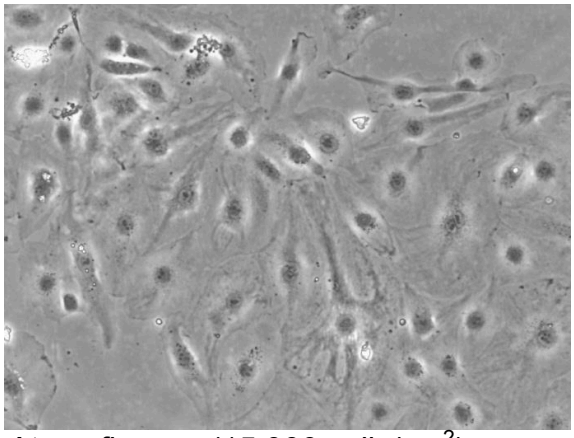


24 hours after plating (5,000 cells/cm^2)



At confluency (15,000 cells/cm^2)

Passaging:

- Passage when the culture reaches: 80-90% confluent
- Population doubling time: ~3 days
- Recommended passaging reagent: Trypsin/EDTA

- Remove culture medium and wash culture vessel with PBS
- Add an appropriate volume of trypsin/EDTA solution to the culture vessel
- Incubate for 3-4 minutes at 37°C until the cells have rounded up
- Gently resuspend the cells in the flask with the spent trypsin/EDTA
- Neutralize the trypsin with **ARE Liver Sinusoidal Endothelial Cell Culture Medium**
- Transfer the cell suspension to a sterile conical tube and centrifuge at 620 *x g* for 5 min
- Carefully remove the supernatant and resuspend in 2 mL of pre-warmed **ARE Liver Sinusoidal Endothelial Cell Culture Medium** and perform a cell count
- Dilute the cells into the required volume of pre-warmed **ARE Liver Sinusoidal Endothelial Cell Culture Medium**
- Seed cells into the culture vessel (coated with Type I Collagen) at the recommended seeding density

Do not passage the cells more than twice as the cells may become senescent and lose their biological functionality.

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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