Assay-Ready Expanded Human Liver Sinusoidal Endothelial Cells

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

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/cm²
- 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
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.