Human MCF7 Breast Cancer Cells

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Product Name</th>
<th>Product Quantity</th>
<th>Storage on Arrival</th>
<th>Thawing Instructions</th>
<th>Storage once Thawed</th>
</tr>
</thead>
<tbody>
<tr>
<td>ax4010</td>
<td>Parental MCF7 Cells (MCF7/S0.5)</td>
<td>1 million cells/vial</td>
<td>Liquid nitrogen</td>
<td>Follow protocol</td>
<td>N/A</td>
</tr>
<tr>
<td>ax4011</td>
<td>Tamoxifen-Resistant MCF7 Cells (MCF7/TAMR-4)</td>
<td>1 million cells/vial</td>
<td>Liquid nitrogen</td>
<td>Follow protocol</td>
<td>N/A</td>
</tr>
<tr>
<td>ax4012</td>
<td>Tamoxifen-Resistant MCF7 Cells (MCF7/TAMR-8)</td>
<td>1 million cells/vial</td>
<td>Liquid nitrogen</td>
<td>Follow protocol</td>
<td>N/A</td>
</tr>
<tr>
<td>ax4013</td>
<td>Fulvestrant-Resistant MCF7 Cells (MCF7/182R-6)</td>
<td>1 million cells/vial</td>
<td>Liquid nitrogen</td>
<td>Follow protocol</td>
<td>N/A</td>
</tr>
<tr>
<td>ax0044</td>
<td>Unlock</td>
<td>25 mL</td>
<td>Thaw at 4°C</td>
<td>Store at 4°C for up to 1 week</td>
<td></td>
</tr>
</tbody>
</table>

Lot-specific information is 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: See culture medium section
- Seeding density (ax4010): 4,000 viable cells/cm²
- Seeding density (ax4011, ax4012, ax4013): 5,600 viable cells/cm²
- Recommended centrifugation speed: 200 x g for 5 minutes

Preparation of Cell Culture Medium:

- Parental MCF7 Cells (ax4010) should be cultured in the following cell culture medium: DMEM/F12 medium (no phenol red) supplemented with 1% fetal bovine serum + 2.5 mM GlutaMAX™ + 6 ng/mL insulin.
**MCF7 Breast Cancer Cell Protocol**

- **Tamoxifen-Resistant MCF7 Cells (ax4011 and ax4012)** should be cultured in the following cell culture medium:
  
  **DMEM/F12 medium (no phenol red) supplemented with 1% fetal bovine serum + 2.5 mM GlutaMAX™ + 6 ng/mL insulin + 1 μM tamoxifen.**

- **Fulvestrant-Resistant MCF7 Cells (ax4013)** should be cultured in the following cell culture medium:
  
  **DMEM/F12 medium (no phenol red) supplemented with 1% fetal bovine serum + 2.5 mM GlutaMAX™ + 6 ng/mL insulin + 100 nM fulvestrant.**

- Tamoxifen and fulvestrant should be added to the cell culture medium immediately prior to use.
- Minimize exposure of the cell culture media to light at all times.

**Thawing & Plating:**

- 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 transfer to a 15 mL sterile conical tube.
- Slowly add 10 mL of pre-warmed, 37°C, cell culture medium.
- Rinse the cryovial with 1 mL of medium to ensure all of the cells are transferred.
- Centrifuge the cells at 200 x g for 5 min.
- Carefully remove the supernatant and resuspend the cell pellet in 1-2 mL of pre-warmed, 37°C, cell culture medium.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed, 37°C, cell culture medium.
- Seed cells into the culture vessel at the recommended seeding density.
- Incubate the cells at 37°C, 5% CO₂ in a humidified incubator.
- Replace the cell culture medium every 2-3 days depending on cell confluency.

**Passaging:**

- Passage when the culture reaches: 80-100% confluent
- Recommended passaging reagent: Unlock

- Remove all spent medium from cell culture vessels.
- Gently rinse the surface of the cell layer once with PBS, 2 mL of PBS per 10 cm² culture surface area. Discard the PBS.
- Add 1 mL per 10 cm² of culture surface area of cold/room temperature Unlock passaging reagent. Evenly distribute it over the entire cell layer.
• 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 passaging reagent with four volumes pre-warmed, 37°C, cell culture medium. For example, if 1 mL of Unlock is used, then add 4 mL of the medium to stop the reaction.
• Transfer the cell suspension to a sterile conical tube.
• Centrifuge the cells at 200 x g for 5 minutes.
• Carefully remove the supernatant and resuspend the cell pellet in 1-2 mL of pre-warmed, 37°C, cell culture medium.
• Perform a cell count to determine the number of viable cells.
• Dilute the cells into the required volume of pre-warmed, 37°C, cell culture medium.
• Seed cells into the culture vessel at the recommended seeding density.
• Incubate the cells at 37°C, 5% CO₂ 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 application to humans.

The end user is not permitted to transfer or re-freeze the cells. It is recommended that low passage cells are used for endpoint experiments.