# Human MCF7 Breast Cancer Cells

| Catalog No. | Product Name | Product Quantity | Storage on Arrival | Thawing Instructions | Storage once Thawed |
|---|---|---|---|---|---|
| ax4010 | Parental MCF7 Cells (MCF7/S0.5) | 1 million cells/vial | Liquid nitrogen | Follow protocol | N/A |
| ax4011 | Tamoxifen-Resistant MCF7 Cells (MCF7/TAMR-4) | 1 million cells/vial | Liquid nitrogen | Follow protocol | N/A |
| ax4012 | Tamoxifen-Resistant MCF7 Cells (MCF7/TAMR-8) | 1 million cells/vial | Liquid nitrogen | Follow protocol | N/A |
| ax4013 | Fulvestrant-Resistant MCF7 Cells (MCF7/182R-6) | 1 million cells/vial | Liquid nitrogen | Follow protocol | N/A |
| ax0044 | Unlock | 25 mL | Aliquot & store at -80°C for up to 6 months | Thaw at 4°C | Store at 4°C for up to 1 week |

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/cm$^2$**
- Seeding density (ax4011, ax4012, ax4013): **5,600 viable cells/cm$^2$**
- 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.**

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- **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% $CO_2$** 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 $cm^2$ culture surface area. Discard the PBS.
- Add 1 mL per 10 $cm^2$ of culture surface area of **cold/room temperature** **Unlock** passaging reagent. Evenly distribute it over the entire cell layer.

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- 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% $CO_2$** 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 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.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
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