MCF7

HTB-22™

Description

Organism: *Homo sapiens*, human
Cell Type: epithelial cell
Tissue: Breast; Mammary gland
Age: 69 years
Gender: Female
Morphology: epithelial
Growth properties: Adherent and/or suspension
Disease: Adenocarcinoma

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local

or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Complete medium:

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium (ATCC 30-2003). To make the complete growth medium, add the following components to the base medium:

1.4 mL Gibco™ Insulin, human recombinant, zinc solution (4 mg/mL stock); Thermofisher catalog # 12585-014

fetal bovine serum to a final concentration of 10%.

Handling Procedure:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. It is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and centrifuge the cell suspension at approximately 150-400 x g for 8-12 minutes (280xg for 10 min). Discard the supernatant and resuspend the cells with recommended growth media (see the specific lot information on the Certificate of Analysis for culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask as recommended on the Certificate of Analysis) at a seeding density of 8.0 x 10^4 to 1.5 x 10^5 viable cells/cm2.
4. Transfer the cell pellet to an appropriate size vessel. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product

sheet.

Note: MCF7 (HTB-22) is typically a slow-growing cell line (will seem like it is not growing) and will appear as loosely attached three-dimensional clusters with some floating viable cells. It can take the HTB-22 cells 6-12 days to get to 80% confluence.

It is not unusual for MCF7 cells to display delayed attachment until after the first or second subculture.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

Note: if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with D-PBS (ATCC 30-2200) to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution (ATCC 30-2101) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37°C.

Note:

The appearance of floating clusters during the first two subcultures is normal. These

clusters should be gently broken up using smallbore pipette (5 mL or smaller). The cells should, after a few days' incubation, reattach as three-dimensional islands (there still be some clusters that do not reattach). Growth will eventually spread out from the islands and the culture should, after the 2nd subculture. flatten and become 70-80% confluent.

Due to the semi-attached nature of this cell the minimum volume per vessel should be used to seed flasks. Until growth spreads out from the islands and the culture flattens. Treat as a semi-attached cell line by adding media as the clusters increase.

Subcultivation Ratio or Subculturing Seeding Density: A subcultivation ratio of 1:3 to 1:6 is recommended or a subculturing seeding density from 4.0 x 10^4 to 1.0 x 10^5 viable cells/cm^2 is recommended
Medium Renewal: 2 to 3 times per week
Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: MCF7 (ATCC HTB-22)

References

References and other information relating to this material are available at www.atcc.org.

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