Product Sheet



# **HTB-20<sup>™</sup>**

# Description

BT-474 is a cell line exhibiting epithelial morphology that was isolated from a solid, invasive ductal carcinoma of the breast obtained from a 60-year-old, White, female breast cancer patient. Use these cells in your cancer research. **Organism:** *Homo sapiens*, human **Tissue:** Breast; Duct; Mammary gland Age: 60 years **Gender:** Female Morphology: epithelial Growth properties: Adherent, patchy Disease: Ductal Carcinoma

# **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.

## BSL1

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



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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<sub>2</sub>

# 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

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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 Hybri-Care Medium, Catalog No. 46-X. Hybri-Care Medium is supplied as a powder and should be reconstituted in 1 L cell-culture-grade water and supplemented with 1.5 g/L sodium bicarbonate. To make the complete growth medium, add the following components to the base medium: 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.

- 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. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x g for 5 to 7 minutes.
- Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm<sup>2</sup> or a 75 cm<sup>2</sup> culture flask.
- 5. Incubate the culture at 37°C in a suitable incubator. A 5%  $\rm CO_2$  in air atmosphere is recommended if using the medium described on this product sheet.

#### Subculturing procedure:

HTB-20 recovers slowly from cryopreservation. It may take two to four weeks for the cells to reach 70-80% confluence in a T-75 flask after thaw.

Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 mL of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37°C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. Corning<sup>®</sup> T-75 flasks (catalog #430641) are recommended for subculturing this product.

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**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 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: BT-474 (ATCC HTB-20)

## References

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

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# Revision

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