

<b>Code:</b>	0353
<b>Cell Line:</b>	BT-474
<b>Species:</b>	Homo sapiens <b>Vulgar Name:</b> Human
<b>Tissue:</b>	Mammary gland; breast/duct
<b>Morphology:</b>	Epithelial
<b>Disease:</b>	Ductal Carcinoma
<b>Growth Properties:</b>	Adherent, patchy (The cells form adherent patches of epithelial-like cells)
<b>Sex:</b>	FEMALE
<b>Age Ethnicity:</b>	60 years adult; caucasian
<b>Derivation:</b>	The BT-474 line was isolated from a solid, invasive ductal carcinoma of the breast.
<b>Tumor Formation:</b>	Yes, in Amsterdam/IMR rats with regression in 10 days. Yes, in nude mice.
<b>Biosafety:</b>	1
<b>Culture Medium:</b>	Hybri-Care Medium from ATCC Catalog No. 46-X. 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%.
<b>Subculturing:</b>	This cells 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. T-75 flasks are recommended for subculturing this product. NOTE: For more information on enzymatic dissociation and subculturing

of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010. "

**Medium Renewal:** 2 to 3 times per week

**Subcultivation ratio:** 1:2 to 1:3 is recommended

**Culture Conditions:** Atmosphere: air, 95%; carbon dioxide (CO<sub>2</sub>), 5% Temperature: 37°C

**Cryopreservation:** 95% FBS + 5% DMSO (Dimethyl sulfoxide)

**Thawing Frozen Cells:** SAFETY PRECAUTION: Is highly recommend that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring 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. For cells that are sensitive to DMSO 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 spin at approximately 125 x g for 5 to 7 minutes.
4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio).
5. Incubate the culture in a appropriate atmosphere and temperature (see ""Culture Conditions"" for this cell line).

NOTE: 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).

**References:** Lasfargues EY, et al. Isolation of two human tumor epithelial cell lines from solid breast carcinomas. J. Natl. Cancer Inst. 61: 967-978, 1978. PubMed: 212572 Lasfargues EY, et al. A human breast

tumor cell line (BT-474) that supports mouse mammary tumor virus replication. In Vitro 15: 723-729, 1979. PubMed: 94035  
Littlewood-Evans AJ, et al. The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. Cancer Res. 57: 5386-5390, 1997. PubMed: 9393764  
The cells form adherent patches of epithelial-like cells The patches are compact multilayered colonies that rarely become confluent Lasfargues EY, et al. Isolation of two human tumor epithelial cell lines from solid breast carcinomas. J. Natl. Cancer Inst. 61: 967-978, 1978. PubMed: 212572

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**ATCC:** HTB-20