BCRJ Code:	0353
Cell Line:	BT-474
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Mammary Gland; Breast/Duct
Morphology:	Epithelial
Disease:	Ductal Carcinoma
Growth Properties:	Adherent, patchy (The cells form adherent patches of epithelial-like cells)
Sex:	Female
Age/Ethinicity:	60 Year /
Derivation:	The BT-474 line was isolated from a solid, invasive ductal carcinoma of the breast.
Tumor Formation::	Yes, in Amsterdam/IMR rats with regression in 10 days Yes, in nude mice
Biosafety:	1
Culture Medium:	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 fetal bovine serum to a final concentration of 10%.

Subculturing:	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.
Subculturing Medium Renewal:	2 to 3 times per week
Subculturing Subcultivation Ratio:	1:2 to 1:3 is recommended
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris. 1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize 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 its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions. 3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes. 4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio). 5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at 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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