Product sheet

BT-474 Cells | 300131

General information

Description	BT-474 is a human breast cancer cell line, derived from the ductal carcinoma of a 60-year-old female. This cell line is estrogen and progesterone receptor positive, making it a valuable model for studying hormone-responsive breast cancers. BT-474 cells are also characterized by the overexpression of HER2/neu (human epidermal growth factor receptor 2), a protein that is amplified and plays a critical role in the pathogenesis and progression of certain aggressive types of breast cancer. The BT-474 cell line is extensively used in oncological research to study the molecular mechanisms of breast cancer proliferation and to test therapeutic strategies targeting hormone receptors and the HER2 pathway. These cells are particularly useful for examining the efficacy of HER2-targeted therapies, such as trastuzumab (Herceptin), and for exploring mechanisms of resistance to these treatments. The cell line has also contributed to advancements in understanding how hormonal manipulations affect cancer cell growth and survival, providing insights into potential treatment approaches for hormone-dependent tumors.
Organism	Human
Tissue	Breast, mammary gland
Disease	Invasive ductal carcinoma
Metastatic site	Ductal
Synonyms	Bt-474, BT474

Characteristics

Age	60 years
Gender	Female
Ethnicity	Caucasian
Morphology	Epithelial-like
Growth properties	The cells grow in compact, slowly growing multi-layered colonies which rarely become confluent. A confluent monolayer is not formed.

Regulatory Data

Citation	BT-474 (Cytion catalog number 300131)

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Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_0179

Biomolecular Data

Receptors expressed	HER-2/NEU+, ER+, PR+
Isoenzymes	G6PD, B, PGM3, 1, PGM1, 1, ES-D, 1, Me-2, 0, AK-1, 1, GLO-1, 1, Phenotype Frequency Product: 0.0426
Tumorigenic	Yes, in nude mice
Virus susceptibility	Mouse mammary tumor virus (RIII-MuMTV)
MSI-status	Stable (MSS)
Mutational profile	TP53 mut
Karyotype	Mode = 55, range = 50 to 112, bimodal shift 58 - 59 and 100 in later passages with 3 marker chromosomes

Handling

Culture Medium	DMEM:Ham's F12 (1:1), w: 3.1 g/L Glucose, w: 2.5 mM L-Glutamine, w: 15 mM HEPES, w: 0.5 mM Sodium pyruvate, w: 1.2 g/L NaHCO3 (Cytion article number 820400a)
Supplements	Supplement the medium with 10% FBS, 10 microgram/mL Insulin
Doubling time	60 to 80 hours
Subculturing	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

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Seeding density	2 x 10^4 cells/cm^2 will yield in a mostly confluent layer in about 4 days
Fluid renewal	2 to 3 times per week
Post-Thaw Recovery	Almost 100% recovered cells at >90% viability
Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.
Thawing and Culturing Cells	1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. 2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required. 3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains. 4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening. 5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently. 6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. 7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth. 8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.
Incubation Atmosphere	37°C, 5% CO_2, humidified atmosphere.
Flask Coating	None

Shipping Conditions	Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.
Storage Conditions	For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

Sterility	Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods. To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.