

BT-474 Cells | 300131

General information

Description	In 1978, the BT-474 line was isolated by E. Lasfargues and W.G. Coutinho from a solid, invasive ductal carcinoma of the breast.
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.

Identifiers / Biosafety / Citation

Citation	BT-474 (Cytion catalog number 300131)
Biosafety level	1

Expression / Mutation

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

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Tumorigenic Yes, in nude mice

Virus susceptibility mouse mammary tumor virus (RIII-MuMTV)

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, w: 3.1 g/L Glucose, w: 1.6 mM L-Glutamine, w: 15 mM HEPES, w: 1.0 mM Sodium pyruvate, w: 1.2 g/L NaHCO₃ (Cytion article number 820400a)

Medium supplements Supplement the medium with 10% FBS, 5 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.

Split ratio A ratio of 1:2 to 1:3 is recommended

Seeding density 2×10^4 cells/cm² will yield in a mostly confluent layer in about 4 days

Fluid renewal 2 to 3 times per week

Freezing recovery Almost 100% recovered cells at >90% viability

Freeze medium CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

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Handling of cryopreserved cultures

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. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
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.

Quality control / Genetic profile / HLA

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.

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STR profile

Amelogenin: x,x
CSF1PO: 10,11
D13S317: 11
D16S539: 9, 11
D5S818: 11, 13
D7S820: 9, 12
TH01: 7
TPOX: 8
vWA: 15, 16
D3S1358: 17
D21S11: 28, 32.2
D18S51: 13, 18
D8S1179: 10, 12
FGA: 22, 25
D1S1656: 13, 15.3
D2S1338: 19
D12S391: 17, 18
D19S433: 14, 17

HLA alleles

A*: 01:01:01, 05:02:01
B*: 07:02:01, 20:03:01
C*: 07:02:01, 16:01:01
DRB1*: 04:01:00, 15:01:00
DQA1*: 01:02:01, 03:03:01
DQB1*: 06:02:01
DPB1*: 04:01:01G, 05:01:01G
E: 01:01:01, 01:03:02