Product sheet

BT-474 | 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

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
Medium supplements	10% FBS, 5 microgram/ml Insulin, 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
Passaging solution	The utilization of a passaging solution is not necessary when passaging cells that are cultured in suspension. The appropriate procedure is to dilute the cells in accordance with the indicated guidelines.
Doubling time	60 to 80 hours
Subculturing	Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10 ml for T75 cell culture flasks). Add Accutase (1-2 ml per T25, 2.5 ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at ambient temperature for 8-10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3 min at 300 g, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
Split ratio	A ratio of 1:2 to 1:3 is recommended
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
Freezing recovery	Almost 100% recovered cells at >90% viability
Freeze medium	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

Handling of cryopreserved cultures	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300 x g for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Resuspend the cells carefully in 10 ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the subculture section.
Handling of proliferating cultures	One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Incubate at 37 degree Celsius for a minimum of 24 hours.

Quality control / Genetic profile / HLA

Sterility	Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.
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