Data Sheet

BCRJ Code:	0386
Cell Line:	HCC70
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Breast; mammary gland/duct
Cell Type:	Epithelial
Morphology:	Epithelial
Disease:	TNM stage IIIA, grade 3, primary ductal carcinoma
Growth Properties:	Adherent, The line grows as attached medium-sized epithelial cells
Sex:	Female
Age/Ethinicity:	49 Year /
Derivation:	The HCC70 cell line was initiated from a primary ductal carcinoma on June 3, 1992, and took 44 months to establish.
Products:	Epithelial glycoprotein 2 [EGP2]; cytokeratin 19
Biosafety:	1
Addtional Info:	The cells are poorly differentiated. The tumor was classified as TNM Stage IIIA, grade 3, invasive ductal carcinoma with metastases in 4 out of 17 lymph nodes. The cells overexpress p53, and are negative for the expression of Her2-neu oncogenes. HCC70 is positive for the epithelial cell specific marker Epithelial Glycoprotein 2 (EGP2) and for cytokeratin 19.
Culture Medium:	RPMI-1640 medium modified to contain 2 mM L-glutamine, 4500 mg/L glucose with fetal bovine serum to a final concentration of 10%.

Subculturing:	Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 1.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Maintain cultures at a cell concentration between 4x104 and 4 x 105 cells/cm2. Place culture vessels in incubators at 37°C. 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:	Every 2 to 3 days
Subculturing Subcultivation Ratio:	1:4 to 1:6
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:	Kao J, et al. Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery. PlosONE 4 (7): e 6146, 2009 Kim MS, et al. Breast cancer diagnosis using a microfluidic multiplexed immunohistochemistry platform. PlosONE 5 (5): e10441, 2010 Gazdar AF, et al. Characterization of paired tumor and non-tumor cell lines established from patients with breast cancer. Int. J. Cancer 78: 766-774, 1998. PubMed: 9833771
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