Data Sheet

BCRJ Code:	0351
Cell Line:	HCC827
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Lung
Cell Type:	Epithelial
Disease:	Adenocarcinoma
Growth Properties:	Adherent
Sex:	Female
Age/Ethinicity:	39 Year /
Biosafety:	1
Addtional Info:	This lung adenocarcinoma has an acquired mutation in the EGFR tyrosine kinase domain (E746 - A750 deletion)
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. An inoculum of 5 x 103 to 7 x 103 viable cells/cm2 is recommended. Place culture vessels in incubators at 37°C. Maintain cultures at a cell concentration between 3 x 10e4 and 5 x 10e4 cells/cm2. Population Doubling Time: 28 hours 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:

Girard L, et al. Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. Cancer Res. 60: 4894-4906, 2000. PubMed: 10987304 Burbee DG, et al. Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. J. Natl. Cancer Inst. 93: 691-699, 2001. PubMed: 11333291 Toyooka S, et al. Differential expression of FEZ1/LZTS1 gene in lung cancers and their cell cultures. Clin. Cancer Res. 8: 2292-2297, 0. PubMed: 12114433 Virmani A, et al. Aberrant methylation of the cyclin D2 promoter in primary small cell, nonsmall cell lung and breast cancers. Int. J. Cancer 107: 341-345, 2003. PubMed: 14506731 Liu CX, et al. LRP-DIT, a putative endocytic receptor gene, is frequently inactivated in non-small cell lung cancer cell lines. Cancer Res. 60: 1961-1967, 2000. PubMed: 10766186

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