

## Data Sheet

PAGE 1/3

<b>BCRJ Code:</b>	0412
<b>Cell Line:</b>	RPMI 2650
<b>Species:</b>	Homo sapiens
<b>Vulgar Name:</b>	Human
<b>Tissue:</b>	Nose; Nasal septum
<b>Cell Type:</b>	Epithelial
<b>Morphology:</b>	Epithelial
<b>Disease:</b>	Squamous Cell Carcinoma
<b>Growth Properties:</b>	Adherent
<b>Sex:</b>	Male
<b>Age/Ethnicity:</b>	52 Year /
<b>Derivation:</b>	From the pleural effusion of an extensive malignant tumour of the nasal septum, diagnosed as anaplastic squamous cell carcinoma. Nearly normal karyotype, stability of chromosome number and probable origin from malignant cells make the cell line unique amongst permanent human cell lines. Cells are very small and grow in clusters fusing to form thick layers.
<b>Virus Susceptibility::</b>	Human poliovirus 1 Herpes simplex virus Vesicular stomatitis, Glasgow (Indiana) Vesicular stomatitis, Orsay (Indiana)
<b>Products:</b>	Genes expressed: mucoid; keratin Isoenzymes: G6PD, B
<b>Biosafety:</b>	1
<b>Culture Medium:</b>	EMEM (EBSS) + 2mM Glutamine + 1% Non Essential Amino Acids (NEAA) + 10% of fetal Bovine Serum.

**Subculturing:**

This cells attach in clusters. Cells will pile and the culture does not get 100% confluent. Volumes used in this protocol are for 75 cm<sup>2</sup> flasks; 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 which contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until 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. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures 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:**

2 to 3 times per week

**Subculturing Subcultivation Ratio:**

1:2 to 1:4

**Culture Conditions:**

Atmosphere: air, 95%; carbon dioxide (CO<sub>2</sub>), 5% Temperature: 37°C

**Cryopreservation:**

95% FBS + 5% DMSO (Dimethyl sulfoxide)

### Thawing Frozen Cells:

**SAFETY PRECAUTION:** It is highly recommended that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris. 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions. 3. For cells that are sensitive to DMSO it is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. 4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 5. Incubate the culture in a appropriate atmosphere and temperature (see "Culture Conditions" for this cell line). **NOTE:** It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

### References:

Exp Cell Res 1965;39:190; Cancer 1964;17:170

### Depositors:

Banco de Células do Rio de Janeiro

### ATCC:

CCL-30