Data Sheet

BCRJ Code:	0366
Cell Line:	PNT-2
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Prostate
Morphology:	Epithelial
Disease:	Normal
Growth Properties:	Adherent
Sex:	Male
Derivation:	Established by immortalisation of normal adult prostatic epithelial cells by transfection with a plasmid containing SV40 genome with a defective replication origin. The primary culture was obtained from a prostate of a 33 year old male at post mortem. PNT2 cells contain the SV40 genome and express large T protein. They possess a well differentiated morphology with the expression of cytokeratin 8,18 and 19 with the latter being a feature of differentiated luminal cells of the glandular prostate. Cytokeratin 14, a marker of epithelial basal cells, is not expressed.
Tumor Formation::	No, in nude mice.
Biosafety:	2
Culture Medium:	RPMI 1640 + 2mM L-glutamine + 10% of fetal bovine serum.

Subculturing:	Split sub-confluent cultures (70-80%). 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. 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 Subcultivation Ratio:	1:3 to 1:10
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO_2), 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:	J Urol 1991;146(3) 881-6; Int J Oncol 1995;6; Int J Cancer 1995;62:724, Biochem Pharm 1999;58: 279-284
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