Data Sheet

BCRJ Code:	0203
Cell Line:	PC-12
Species:	*Rattus norvegicus*
Vulgar Name:	Rat
Tissue:	Adrenal Gland
Morphology:	Small Irregularly Shaped Cells
Disease:	Pheochromocytoma
Growth Properties:	Floating Clusters; Few Scattered Lightly Attached Cells.
Sex:	Male
Applications:	This cell line is a suitable transfection host.
Tumor Formation::	Yes, in New England Deaconess Hospital strain rats
Products:	Catecholamines; dopamine; norepinephrine
Biosafety:	1
Addtional Info:	The cells respond reversibly to NGF by induction of the neuronal phenotype when plated on Collagen IV coated culture flasks. The cells do not synthesize epinephrine.
Culture Medium:	RPMI 1640 with 2.0 mM L-glutamine, 4.5 g/L glucose, 1.0 mM sodium pyruvate, heat-inactivated horse serum to a final concentration of 10% and fetal bovine serum to a final concentration of 5%.

Subculturing:	Protocol: Volumes used for this protocol are for a 75cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. T-75 flasks are recommended for subculturing this product. Transfer cell suspension to centrifuge tube. Centrifuge cells at 180 to 225 xg for 8-15 minutes at room temperature. Remove and discard supernatant leaving cell pellet. Resuspend the cell pellet with 5 mls of fresh medium (or use an appropriate volume of medium which is a multiple of 5 to facilitate the next step). Gently aspirate each 5 ml aliquot of cells 4 or 5 times with a new 20 ml syringe outfitted with a 22g (1½ in.) needle to break up cell clusters. Add appropriate aliquots of the cell suspension to new 75 cm2 flask with 10-15 ml fresh growth medium. Seed flask 5 x 10(5) to 1 x 10(6) viable cells/ml or use subcultivation ratio of 1:2 to 1:4. Place culture vessels in incubator at 37ºC Subculture when cell density reaches between 2-4 x 10(6) viable cells/ml. 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
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:	Levi A et al. Molecular cloning of gene sequence regulated by nerve growth factor. Science 229:393-395, 1985 Pub Med: 85244666
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