Cell Line Designation: PC-12 cells
AddexBio Catalog No. C0032001

Cell Line Description:

Disease: Rat adrenal pheochromocytoma

Species: Rat, Rattus norvegicus

Tissue: Adrenal gland

Properties: Suspension, floating clusters, few scattered loosely attached cells

Cytogenic data: 40 chromosomes, 38 autosomes plus XY

Complete Medium: AddexBio formulated RPMI-1640 Medium (C0004-01) + 10% Horse Serum + 5% FBS

Subculture Procedure: The cells do grow satisfactorily in suspension in untreated flasks. Maintain cultures between 2-5x100,000 cells/ml. For attachment grow in collagen coated flasks (must be collagen type IV), feed 3 times a week and split cultures 1:3 to 1:6 (i.e. seeding at 2-4 x 10,000 cells / cm2) using 0.25% trypsin/EDTA. Culture at 5% CO_2, 37°C.

Growing cultures will be supplied in suspension. On receipt, incubate the flask overnight without opening and on introduction to a type IV collagen coated flask the cells should attach. Preparation of collagen solution: Use collagen type IV (Sigma C5533). Add 5mg collagen to 50ml 0.1M glacial acetic acid to obtain a 0.01% collagen solution. Stir at room temperature for 1-3 hours. Store in sterile glass bottles or as pre-coated flasks at 4°C. NB: Growth factor reduced matrigel may also be used. Preparation of flasks: Add sufficient collagen to cover the surface of the required number of tissue culture flasks and leave for at least 6 hours at 37°C. Remove excess fluid and allow flasks to dry by incubating at 37°C, leaving the caps loose. Prior to addition of cells wash flask three times in PBS. Flasks can be purchased from suppliers pre-coated with type IV collagen. Reported doubling time = 92 hours

Medium Renewal: Two to three times weekly.

Freezing Medium: Complete culture medium supplemented with 5% (v/v) DMSO

Additional Information: Additional product and technical information can be obtained from the catalog references and the Addexbio Technical Information site at www.addexbio.com, or by email at customersupport@addexbio.com.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafty/bmbl4/bmbl4toc.htm

Use Restrictions: **These cells are distributed for research purposes only.** Addexbio does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Procedure for Frozen Cells:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

Safety Precaution:

Addexbio highly recommends 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 submersed 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 O-ring and cap out of 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. Transfer the vial contents to the centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125xg for 5 to 7 minutes. (Optional if one wants to remove DMSO)

4. Resuspend cell pellet with the recommended complete medium and dispense into a new culture flask. 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 complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

5. One may also transfer the vial contents into a new culture flask if removal of DMSO is not important. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

6. Incubate the culture at 37°C in a suitable incubator for 24-48 hours for cell attachment. A 5% CO_2 in air atmosphere is recommended.

Handling Procedure for Cells in Flask Culture:

The flask was seeded with cells grown and completely filled with complete medium at AddexBio facility that acts as a cushion and to prevent loss of cells during shipping.

1. Upon receipt, carefully examine if the majority of the cells are attached to the bottom of the flask using an inverted microscope (preferably equipped with phase-contrast optics), as the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable) during shipping. In addition, visually examine the culture for macroscopic evidence of any microbial contamination.
2. **If the cells are still attached**, asceptically remove all but 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, asceptically remove the entire contents of the flask and centrifuge at 125 x g for 5 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to a 25 cm^2 flask (T25). Incubate at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

References for PC-12 cells:

1. Levi A, Eldridge JD, Paterson BM. Molecular cloning of a gene sequence regulated by nerve growth factor. Science. 1985 Jul 26;229(4711):393-395.

Lot Specific Information Sheet for AddexBio Cat #: C0032001

Lot Number: 0893759

Designation: PC-12 cells

Total Cells/mL: >$1.2x10^6$

Expected Viability: 70.0-75.1%

Ampule Passage #: 6

Dilute Ampule Content: 1:10 (T-25)

Volume/Ampule: 1 mL

A T-25 setup at a seeding density of $3.7x10^5$ viable cells/mL is ready to subculture in 5 days.

A T-75 setup at a seeding density of 5.0×10^5 viable cells/mL is ready to subculture in 6 days.