

PC-12 Cell Line Protocol

General Information:

- Cells were purchased from ATCC (Catalog # CRL-1721)
- Cells are stored in liquid N2 tank (Rack 1 Box A)

Culturing & Maintenance:

- Remove cells from N2 and rapidly thaw in a 37°C waterbath with constant agitation (Should take about 2 minutes)
- Once thawed, transfer the aliquot into a 15 mL conical tube containing 9 mL of Complete Culture Medium
- Spin at 12500 RPM for 5 minutes
- Remove supernatant and re-suspend the cell pellet in 15 mL of fresh Complete Culture Medium in a 60 mm
- Feed cells every other day with a 100% change into fresh Complete Culture Medium

Carrying / Plating:

- Once cells are about 80% confluent, aspirate off the culture media and add back 10 mL of fresh Complete Medium
- Use a cell scraper to gently lift cells from the plate
- Triturate with a glass Pasteur Pipette to break up any chunks and plate into new 60 mm dishes to carry, or into experimental dishes
 - To carry, cells should be diluted to anywhere between 1×10^4 cells/mL and 3×10^4 cells/mL
 - Normally the cell count (via hemocytometer) after scraping / triturating is around 18 which is 18×10^4 cells/mL
 - $(18 \times 10^4 \text{ cells/mL})(x) = (1.5 \times 10^4 \text{ cells/mL})(15 \text{ mL})$
 - $x = 1.25 \text{ mL} \rightarrow$ So I will add 1.25 mL of my cell mix to 13.75 mL of complete culture medium in a new 60 mm dish
 - For experiments, the density you plate at depends on the assay, etc...

Differentiating:

- Remove Complete Culture Medium and replace with differentiating medium
 - DMEM containing 1% horse serum, 1% Pen-Strep and 100ng / mL NGF
- Feed cells every other day with differentiating medium until ready for experiment

Medias:

Complete Culture Medium (500 mL):

- 445 mL of DMEM
- 25 mL of horse serum
- 25 mL of calf serum
- 5 mL Pen-Strep

Differentiating Medium (50 mL):

- 48.5 mL of DMEM
- 500 uL NGF
- 500 uL Horse Serum
- 500 uL of Pen-Strep

Product Ordering Information:

PC-12 Cells, CRL-1721, ATCC
DMEM (High glucose), 11965, Invitrogen
Horse Serum, S12159, Atlanta Biologicals
Calf Serum, J0016, Atlanta Biologicals

Pen-Strep, 15140, Invitrogen
7S-NGF, N0513, Sigma