PNEC30

CRL-2930™

Description

PNEC30 is a neuroendocrine cell line that was isolated in 2002 from the prostate of a 6-month-old male mouse. The cell line was established from CR2-TAg prostate tumors and metastases. It can be used for genetic and/or pharmacologic studies of the regulation of NE cell proliferation, differentiation, and tumorigenesis.

Organism: *Mus musculus*, transgenic, mouse, transgenic
Cell Type: neuroendocrine cell
Tissue: Prostate
Age: 6 months
Gender: Male
Morphology: neuronal
Growth properties: Adherent
Disease: Prostate Cancer; Neuroendocrine

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as

guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Complete medium: The base medium for this cell line is Neural Progenitor Basal Medium, which is supplied as part of the NPMM Neural Progenitor Maintenance Medium Bullet Kit available from Lonza/Clonetics Inc., Catalog No. CC-3209. To make the complete growth medium, add the following components to 500 ml of the base medium:

- additives that are supplied with the kit (ATCC does not use gentamycin-amphotericin B)
- heat-inactivated fetal bovine serum (FBS) to a final concentration of 10%
- bovine pituitary extract (BPE) (Lonza/Clonetics, Inc., Catalog No. CC-4009) to a final concentration of 0.3%

Note: Do not filter complete medium.

Handling Procedure:

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.

Note: These cells are cultured on **poly-D-lysine** coated vessels (BD BioCoat® Cellware, BD Biosciences, Cat. No. 356524 for 75 cm^2 flasks) which are additionally coated with 20 µg/mL **laminin** (Sigma, Cat. No. L2020 or equivalent). Add 5 mL laminin solution to a 75 cm^2 flask and incubate overnight at room temperature. Remove laminin solution and allow flask to air dry uncapped and standing upright in a biological cabinet before introducing cells.

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 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. 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 10 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into the appropriate size poly-D-lysine BioCoatÒ Cellware culture flask pre-coated with laminin. 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 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2. flasks; proportionally reduce or increase amount of solutions for culture vessels of other sizes.

Note: These cells are cultured on flasks coated with 0.1 mg/mL Poly-L-Lysine (Sigma catalog # P4707 or equivalent), and then additionally coated with 20 µg/mL of Laminin (Sigma catalog # L2020 or equivalent). Add 5 mL Poly-L-Lysine solution to a 75 cm2 flask and gently rock the flask for 1 to 3 hours at room temperature. Remove the Poly-L-Lysine solution and allow flask to air dry uncapped in a biological safety cabinet. When dry, add 5 mL Laminin solution to the flask and gently rock the flask for 1 to 3 hours at room temperature. Remove the Laminin solution and allow flask to air dry uncapped in a biological safety cabinet before introducing cells. It is recommended to coat the vessel immediately prior to use.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. 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.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 150 to 400 x g for 8 to 12 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new poly-L-lysine and laminin coated culture vessels. An inoculum of 2 X 10^4 to 5 X 10^4 viable cells/cm^2 is recommended.
7. Incubate cultures at 37°C. subculture when cultures reach a cell concentration between 1 X 10^5 and 2 X 10^5 cells/cm^2

Subcultivation ratio: A subcultivation ratio of 1:3 to 1:8 is recommended.
Medium renewal: Every 2 to 3 days.
Reagents for cryopreservation: Complete Culture Medium + 5% DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: PNEC30 (ATCC CRL-2930)

References

References and other information relating to this material are available at www.atcc.org.

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Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor