ATCC

Product Sheet

PNEC30 (ATCC® CRL-2930™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

Intended Use

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

Complete Growth 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.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: PNEC30 (ATCC® CRL-2930™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Mus musculus*, transgenic, mouse, transgenic
Tissue: prostate
Disease: prostate neuroendocrine cancer
Cell Type: neuroendocrine
Age: 6 months
Gender: male
Morphology: neuronal
Growth Properties: adherent

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC 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.

Unpacking & 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.

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.

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.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information), grown, and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and

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many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 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,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to original 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

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 poly-D-lysine coated vessels (BD BioCoat Cellware, BD Biosciences, Cat. No. 356524 for 75 cm^2. flask) 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. 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 125 x g for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new poly-D-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.

Cryopreservation Medium

Fetal bovine serum (FBS), 90%; DMSO, 10%
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.
Fetal bovine serum is available as ATCC® Catalog No. 30-2020.

Comments

GeneChip analyses of cell lines harvested at different passages, and as xenografted tumors, indicated that PNECs express consistent features *ex vivo* and *in vivo* and share a remarkable degree of similarity with primary CR2-TAg prostate neuroendocrine (NE) tumors.
PNECs express *mAsh1* (mouse homolog proneural gene complex *achaete-scute*), a basic helix-loop-helix (bHLH) transcription factor essential for NE cell differentiation in other tissues. PNEC cell lines should be useful for genetic and/or pharmacologic studies of the regulation of NE cell proliferation, differentiation, and tumorigenesis.
PNEC30 cells, when cultured on non-coated surfaces, grow in suspension as multicellular aggregates that resemble the neurospheres of cultured neural stem cells.

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in

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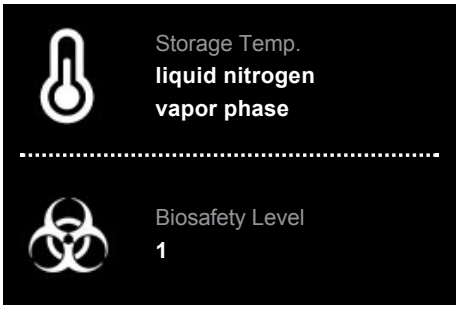

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the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.