Py230

CRL-3279™

Description

Py230 is an epithelial-like cell line that was isolated in 2004 from the mammary gland of an adult female mouse with adenocarcinoma. This cell line was deposited by L Ellies.

Organism: *Mus musculus*, mouse
Cell Type: epithelial-like cell
Tissue: Breast; Mammary gland
Age: adult
Gender: Female
Morphology: epithelial-like
Growth properties: Adherent
Disease: Adenocarcinoma
Volume: 1.0 mL

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 F-12K Medium (ATCC 30-2004). To make the complete growth medium, add the following component to the 500 mL of the base medium:

- Fetal bovine serum (FBS; ATCC 30-2020) for a final concentration of 5%
- MITO+ Serum Extender (Corning® #355006) for a final concentration of 0.1%

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.

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 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 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). 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 sheet.

Subculturing procedure:

Note: these cells will not maintain their differentiation properties without MITO+

Serum Extender.

Note: these cells grow slowly

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

1. Remove and discard culture medium. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) (ATCC 30-2200) or 0.25% Trypsin – 2.21mM EDTA in HBSS (Corning cat no. 25-053-Cl) solution to remove all traces of serum which contains trypsin inhibitor.

2. 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: These cells will form small clumps upon trypsinization.They do not form a single cell suspension. 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.

3. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. An inoculum of 3 X 10^4 to 5 X 10^4 viable cells/cm^2 is recommended.

4. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. Subculture when cell density reaches between 2 X 10^5 and 3 X 10^5 cells/cm^2.

Subcultivation Ratio: 1:3 to 1:6 is recommended.

Medium renewal: every other day

Note: These cells will differentiate if maintained at low density.

Note: It is recommended to make clones from early passages. Subclone by plating into ultra-low adhesion plates at low density for 2 days. Lightly trypsinize cells and pick single cells, place into wells of 96-well plate with 150µL complete medium. Leave in incubator for 2 weeks. Check for colony growth. Keep only colonies that grow and form domes.
Reagents for cryopreservation: Complete growth medium supplemented with 40% (v/v) FBS (ATCC 30-2020) and 10% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Py230 (ATCC CRL-3279)

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