ATCC

Product Sheet

ProPak-X.36 [PP-X.36] (ATCC® CRL-12007™)

Please read this FIRST

Biosafety Level
2

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 ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: ProPak-X.36 [PP-X.36] (ATCC® CRL-12007™)

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: *Homo sapiens*, human
Disease: Leukemia
Age: embryo
Growth Properties: adherent
DNA Profile:
Amelogenin: X
CSF1PO: 11,12
D13S317: 12,14
D16S539: 9
D5S818: 8,9
D7S820: 11,12
THO1: 7,9.3
TPOX: 11
vWA: 16,19

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

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.

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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. It is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 xg for 5 to7 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.
4. 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 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 sheet.

Handling Procedure for Flask Cultures

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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.

4. 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 many of the cells often detach and become suspended in the culture medium (but are still viable).
5. **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.
6. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 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

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:10 is recommended
Medium Renewal: Every 2 to 3 days
Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach.
Add fresh culture medium, aspirate and dispense into new culture flasks.

Cryopreservation Medium

Cryoprotectant Medium
Complete culture medium described above supplemented with an additional 10% fetal bovine serum and 10% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

The ProPak packaging cell lines produce either murine leukemia virus (MLV) xenotropic particles (ProPak-X cells; ATCC CRL-12007) or amphotropic particles (ProPak-A cells; ATCC CRL-12006 and ATCC CRL-12479). They were derived from the human embryonic kidney line, 293 (see ATCC CRL-1573).
To construct the ProPak-X and the ProPak-A-52 cell lines, the ATG in the splice donor/splice acceptor of pCMV plasmid was mutated to ACG.
The CMV promoter was excised (EcoRI/XhoI, blunt-ended), and replaced with the MoMLV LTR (Asp 718/HindIII, blunt-ended) from plasmid pVH2.
The beta-galactosidase gene was replaced by the gag-pol ORF (NotI fragment) to generate pMoMLVgp. pMoMLVgp was co-transfected with pHA58 into 293 cells by calcium phosphate co-precipitation and hygromycin B-resistant cells were selected.
Clones were screened for the level of Gag secretion and one clone secreting high levels of Gag was selected (designated ProGag); this clone yielded high viral titers in transient transfection.
The expression plasmid containing the murine xenotropic env gene, pCI-Ex, was co-transfected with pSV2pac into the ProGag cell line by calcium phosphate precipitation and puromycin-resistant cells were selected.
The resulting cells were screened for Env expression and clones designated ProPak-X, expressing high levels of Env were screened for ability to produce transducing vector. Clone 36 designated ProPak-X.36 was deposited as CRL-12007.
ProPak-based producer cells were demonstrated to be free of replication-competent retrovirus (RCR) by stringent testing.
Consistently higher transduction of target cells was achieved with ProPak-derived amphotropic vector than with PA317-packaged amphotropic vector.
The highest transduction of human hematopoietic progenitor cells was achieved with vector supernatant generated from a coculture of the ProPak-X and ProPak-A cell lines.

References

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

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

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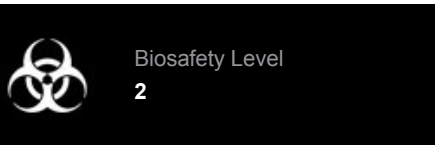

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the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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.

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