ATCC

Product Sheet

Clone C (ATCC® CRL-2531™)

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 DMEM:F12 Medium Catalog No. 30-2006.To make the complete growth medium, add the following components to the base medium:

- 0.005 mg/ml insulin
- 0.005 mg/ml transferrin
- 1.75 ng/ml selenious acid
- 15 ng/ml epidermal growth factor(do not filter)
- 25 ng/ml hydrocortisone
- 10% fetal bovine serum

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Clone C (ATCC® CRL-2531™)

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: *Oryctolagus cuniculus*, rabbit
Cell Type: intercalated; SV40 large T antigen transfecte
Morphology: epithelial
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

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.
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.
1. Thaw the vial by gentle agitation in a **32°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 the cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask.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 **32°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

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 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 **32°C** in a 5% $CO_{2\ in}$ air atmosphere until they

ATCC

Product Sheet

Clone C (ATCC® CRL-2531™)

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 DMEM:F12 Medium Catalog No. 30-2006.To make the complete growth medium, add the following components to the base medium:

- 0.005 mg/ml insulin
- 0.005 mg/ml transferrin
- 1.75 ng/ml selenious acid
- 15 ng/ml epidermal growth factor(do not filter)
- 25 ng/ml hydrocortisone
- 10% fetal bovine serum

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Clone C (ATCC® CRL-2531™)

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

are ready to be subcultured.

3. **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 **32°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 1:6 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 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 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

Primary cultures of rabbit, bicarbonate-secreting, intercalated cells were isolated.
Cells were transfected by electroporation with the pZipSVtsA58 plasmid encoding a temperature-sensitive large T antigen of SV40 plus the neomycin resistance gene under the control of an SV40 promoter.
Following transfection, a line of G418 resistant cells was obtained and designated IC250. These cells were subcloned (Clone C) and characterized.
The cells divide continuously at the permissive temperature of 32C. At the restrictive temperature of 40C, they cease dividing and assume morphological and transport properties of true bicarbonate-secreting intercalated epithelia cells.
They express appropriate ultrastructural features, bind peanut lectin in an apical pattern, are rich in carbonic anhydrase and stain for proton-adenosinetriphosphatase in a basolateral pattern.
These cells reverse the polarity of several proteins and show different cytoskeltal organization depending on the seeding density.
The cell line may be used to study terminal differentiation of intercalated cells.

References

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

Biosafety Level: 2

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

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. 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 strain. 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.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort

Product Sheet

Clone C (ATCC® CRL-2531™)

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 DMEM:F12 Medium Catalog No. 30-2006.To make the complete growth medium, add the following components to the base medium:

- 0.005 mg/ml insulin
- 0.005 mg/ml transferrin
- 1.75 ng/ml selenious acid
- 15 ng/ml epidermal growth factor(do not filter)
- 25 ng/ml hydrocortisone
- 10% fetal bovine serum

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Clone C (ATCC® CRL-2531™)

is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.
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.
© ATCC 2013. All rights reserved. ATCC is a registered trademark of the American Type Culture Collection. [02/03]

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