ATCC

Product Sheet

# CP-B (CP-52731) (ATCC® CRL-4028™)

**Please read this FIRST**

Storage Temp.
**Liquid nitrogen vapor phase**

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 MCDB-153. To make the complete growth medium, add the following components to the base medium:

- 0.4 µg/ml hydrocortisone
- 20 ng/ml recombinant human EGF (Epidermal Growth Factor)
- 8.4 µg/L cholera toxin
- 20 mg/L adenine
- 140 µg/ml BPE (Bovine Pituitary Extract)
- 1x ITS Supplement (Sigma; I1884) [5 µg/ml Insulin; 5 µg /ml Transferrin; 5 ng/ml Sodium Selenite; final concs.]
- 4 mM glutamine
- fetal bovine serum to a final concentration of 5%

Note: To prepare Cholera toxin (Stock 100 µg/mL) : 0.5 mg Cholera toxin (Sigma C8052) + 5 mL $dH_20$. Aliquot into microcentrifuge tubes. Add 84 µL of this 100 µg/mL stock solution to 1L of MCDB-153 base 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: CP-B (CP-52731) (ATCC® CRL-4028™)

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
**Immortalization Method:** hTERT expression
**Tissue:** Esophagus; epithelium
**Disease:** High-grade dysplasia, Barrett's esophagus
**Cell Type:** Epithelial cells immortalized with hTERT
**Age:** Adult
**Gender:** Male
**Morphology:** Epithelial-like
**Growth Properties:** Adherent
**DNA Profile:**
CSF1PO: 8, 12
D13S317: 8, 12
D16S539: 10, 13
D5S818: 11, 12
D7S820: 11, 12
THO1: 8, 9
TPOX: 8, 9
vWA: 18, 20
Amelogenin: XY
**Cytogenetic Analysis:** This is a hypodiploid cell line of male origin with the following derivative chromosomes consistently present at different passages: der(1)t(1;17)(q42?;q21), add(8)(p11.2), der(9)t(9;14)(q10;q10), add(12)(q13), add(15)(q24.3), add(17)(p11.2), del(19)(p13.1), del(21)(q22.1). It should be noted that the tetraploid population was essentially a duplicate of the hypodiploid population and may range from around 18% at lower passages to as high as 50% at higher passages.

## 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 ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor phase and NOT at –70°C. Storage at –70°C will result in loss of viability.

1. Prepare a 25 $cm^2$ or a 75 $cm^2$ culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
2. 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).
3. 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 operations from this point on should be carried out under strict aseptic conditions.
4. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
5. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
6. Incubate the culture at 37°C in a suitable incubator.
7. A 5% $CO_2$/95% air atmosphere is recommended if using the medium described on this product sheet.

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## Handling Procedure for Flask Cultures

The flask was seeded with cells, incubated, and completely filled with medium at ATCC to prevent loss of cells during shipping. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination.

1. 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 37°C with 5% $CO_2$ 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 25-$cm^2$ flask. Incubate at 37°C with 5% $CO_2$ until cells are ready to be subcultured.

## Population Doubling Capacity

As part of our quality control, we have tested this cell line for its ability to grow for a minimum of 15 population doublings after recovery from cryopreservation. We have also compared its karyotype, telomerase expression level, growth rate, morphology and tissue-specific markers when first recovered from cryopreservation with that of cells at 10+ population doublings to ensure that there is no change in these parameters and that the cells are capable of extended proliferation.

## Subculturing Procedure

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.
2. Add 2.0 to 3.0 mL of 0.25% (w/v) Trypsin - 0.53 mM 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 37.0°C to facilitate dispersal.
3. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
4. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant.
5. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 2 X $10^4$ to 3 X $10^4$ viable cells/$cm^2$ is recommended.
6. Incubate cultures at 37°C. Subculture when cells reach a concentration between 1 X $10^5$ and 2 X $10^5$ cells/$cm^2$.

**Subcultivation ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended.
**Medium renewal:** every 3 to 4 days
**Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in Culture Of Aminal Cells: A Manual of Basic Techniques by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

## Cryopreservation Medium

RPMI-1640 Medium, 80%; fetal bovine serum, 10%; DMSO, 10%
Store in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

## Comments

This cell line shows increased telomerase activity and long telomeres of about 12 kb as assessed by terminal restriction fragment lengths (TRF) analysis. Morphologically, the cells are similar to early passage cultures: smaller cells with a larger nucleus to cytoplasm ratio. Genetic instability studies using flow cytometry and FISH reveal the retention of elevated tetraploidy (G2/tetraploidy) in the hTERT-immortalized cells, similar to the non-transduced parental cells.

## References

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

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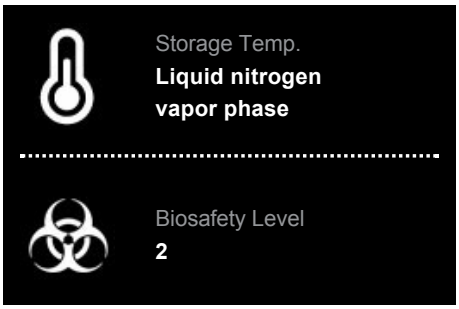


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## Biosafety Level: 2

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