

## Data Sheet

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<b>BCRJ Code:</b>	0270
<b>Cell Line:</b>	C2BBE1 [clone of Caco-2]
<b>Species:</b>	Homo sapiens
<b>Vulgar Name:</b>	Human
<b>Tissue:</b>	Colon
<b>Cell Type:</b>	Enterocyte
<b>Morphology:</b>	Epithelial
<b>Disease:</b>	Colorectal Adenocarcinoma
<b>Growth Properties:</b>	Adherent
<b>Sex:</b>	Male
<b>Age/Ethnicity:</b>	72 Year / Caucasian
<b>Derivation:</b>	The C2BBE1 (brush border expressing) cell line was cloned in 1988 from the Caco-2 cell line by limiting dilution. The clone was selected on the basis of morphological homogeneity and exclusive apical villin localization.
<b>DNA Profile:</b>	Amelogenin: X CSF1PO: 11 D13S317: 11, 13, 14 D16S539: 12, 13 D5S818: 12, 13 D7S820: 11, 12 TH01: 6 TPOX: 9, 11 vWA: 16, 18
<b>Biosafety:</b>	1

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### Additional Info:

The clone was selected on the basis of morphological homogeneity and exclusive apical villin localization. C2BBE1 cells form a polarized monolayer with an apical brush border (BB) morphologically comparable to that of the human colon. Isolated BB contained the microvillar proteins villin, fimbrin, sucrase-isomaltase, BB myosin-1 and the terminal web proteins fodrin and myosin II. The cells express substantial levels of BB myosin I similar to that of the human enterocyte. Although clonal, and far more homogeneous than the parental Caco-2 cell line with respect to BB expression, these cells are still heterogenous for microvillar length, microvillar aggregation, and levels of expression of certain BB proteins; Receptors expressed : epidermal growth factor (Egf)

### Culture Medium:

Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, 0.01 mg/mL human transferrin and 10% of fetal bovine serum.

### Subculturing:

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 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. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. Subculture at 85% of confluence (about every 5 to 7 days). NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

### Subculturing Medium Renewal:

Twice per week

### Subculturing Subcultivation Ratio:

1:6 to 1:10 is recommended

### Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO<sub>2</sub>), 5% Temperature: 37°C

### Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)



**SAFETY PRECAUTION:** It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize 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 its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately  $125 \times g$  for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

**NOTE:** It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

## Thawing Frozen Cells:

## References:

Basson MD, et al. Effect of tyrosine kinase inhibition on basal and epidermal growth factor-stimulated human Caco-2 enterocyte sheet migration and proliferation. J. Cell. Physiol. 160: 491-501, 1994. ; Peterson MD, Mooseker MS. Characterization of the enterocyte-like brush border cytoskeleton of the C2BBE clones of the human intestinal cell line, Caco-2. J. Cell Sci. 102: 581-600, 1992.

## Depositors:

LÚCIO MENDES CABRAL - Universidade Federal do Rio de Janeiro

## Cellosaurus:

[CVCL\\_1096](#)