

## Data Sheet

PAGE 1/3

<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 THO1: 6 TPOX: 9, 11 vWA: 16, 18
<b>Biosafety:</b>	1

## Data Sheet

PAGE 2/3

### 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)



**Thawing Frozen Cells:**

**SAFETY PRECAUTION:** Is highly recommend 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 37°C water bath. To reduce the possibility of contamination, keep the Oring 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. For cells that are sensitive to DMSO 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 x g for 5 to 7 minutes. 4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 5. Incubate the culture in a appropriate atmosphere and temperature (see "Culture Conditions" for this cell line). **NOTE:** 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).

**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:**

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**ATCC:**

CRL-2102