3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

CompoZr® ADME/Tox Cell Lines
C2BBe1 Cells MDR1 –/–/–/–

Catalog Number **MTOX1001**
Storage Temperature –196 °C (liquid nitrogen)

TECHNICAL BULLETIN

Product Description

CompoZr zinc finger nuclease (ZFN) technology is a fast and reliable way to manipulate the genome in a targeted fashion. ZFNs are synthetic proteins engineered to bind DNA at a sequence-specific location and create a double strand break (www.compozrzfn.com). The cell's natural machinery repairs the break in one of two ways: non-homologous end joining or homologous recombination. The non-homologous end joining pathway resulted in modifications at the MDR1 locus (see Figure 1). Single cell knockout clones were isolated and followed for more than twenty passages to establish stable cell lines.

ZFN-mediated gene knockout technology is not limited to diploid targets, allowing the researcher to pursue many of the polyploid cell lines often characteristic of cancer. The colon adenocarcinoma cell line C2BBe1 presents unique challenges to knockout technology as this cell line is tetraploid at the MDR1 locus. Modified cell lines provide the basis for the development of various assays for compound screening. Here, the target gene and corresponding transporter function are eliminated, in contrast to cell lines with normal expression.

ATP-binding cassette (ABC) transporters are a family of transmembrane proteins that utilize ATP hydrolysis for translocation of substrates across membranes. ABC transporters are known to play a critical role in the development of multidrug resistance. Evaluation of membrane transporter pharmacology in drug disposition and drug-drug interactions (DDI) is critical to the pharmaceutical industry's safety evaluation of new drug entities. With this goal in mind, the ABC transporter MDR1 was selected for knock out. Selection was based on the considerable body of evidence supporting its crucial role in efflux across biological membranes of a broad range of therapeutic drugs.[1]

For further information and to download sequence of modified locus, go to the website:
www.wherebiobegins.com/biocells

Components

C2BBe1 mutant cell line with MDR1 gene knocked out, Catalog No. MTOX1001 vial contains ~2 × 10^6 modified C2BBe1 cells	1 vial

The cryoprotectant medium used is Cell Freezing Medium-DMSO 1×, Catalog No. C6164.

Figure 1.
Creation of MDR1 Knockout in C2BBe1 Cells

Site-specific deletion and insertion at the MDR1 Locus in C2BBe1 cell line.

Allele 1– 2 bp deletion:
GGAATTGGTGACAAAATTGGAATGTTCTTTCAGTC
AATGGCAACATTTTTCACTGGGTTTATAGTAGGATT
TACACGTGGTTGGAAGCTAACCCTTGTGATTTTGG
CCATCA**GTCCTGTTCTTGGAC**tgtca**GCTGCTGTCTG**
GGCAAAGGTAGGTGAAGCCTGTGAATCCAGATTTT
GAACTGCACCTTCTCCCTTCCTGCTCTCACCCTAC
GGAAAGGTCTTTTTACTACATGATGACTCTGATTCT
GTACTTGTTACTTTTACGTTTTCTGTTCAAAATCAGC
TGGAT**AAGCATATCCAGCCTCACTGA**

Allele 2– 4 bp deletion:
GGAATTGGTGACAAAATTGGAATGTTCTTTCAGTC
AATGGCAACATTTTTCACTGGGTTTATAGTAGGATT
TACACGTGGTTGGAAGCTAACCCTTGTGATTTTGG
CCATCA**GTCCTGTTCTTGGAC**tgtca**GCTGCTGTCTG**
GGCAAAGGTAGGTGAAGCCTGTGAATCCAGATTTT
GAACTGCACCTTCTCCCTTCCTGCTCTCACCCTAC
GGAAAGGTCTTTTTACTACATGATGACTCTGATTCT
GTACTTGTTACTTTTACGTTTTCTGTTCAAAATCAGC
TGGAT**AAGCATATCCAGCCTCACTGA**

Allele 3– 5 bp deletion:
GGAATTGGTGACAAAATTGGAATGTTCTTTCAGTC
AATGGCAACATTTTTCACTGGGTTTATAGTAGGATT
TACACGTGGTTGGAAGCTAACCCTTGTGATTTTGG
CCATCA**GTCCTGTTCTTGGAC**tgtca**GCTGCTGTCTG**
GGCAAAGGTAGGTGAAGCCTGTGAATCCAGATTTT
GAACTGCACCTTCTCCCTTCCTGCTCTCACCCTAC
GGAAAGGTCTTTTTACTACATGATGACTCTGATTCT
GTACTTGTTACTTTTACGTTTTCTGTTCAAAATCAGC
TGGAT**AAGCATATCCAGCCTCACTGA**

Allele 4– 9 bp deletion and 1 bp insertion:
GGAATTGGTGACAAAATTGGAATGTTCTTTCAGTC
AATGGCAACATTTTTCACTGGGTTTATAGTAGGATT
TACACGTGGTTGGAAGCTAACCCTTGTGATTTTGG
CCATCA**GTCCTGTTCTTGGAC**tgtc**a**a**GCTGCTGTCT**
GGGCAAAGGTAGGTGAAGCCTGTGAATCCAGATTT
TGAACTGCACCTTCTCCCTTCCTGCTCTCACCCTAC
GGAAAGGTCTTTTTACTACATGATGACTCTGATTCT
GTACTTGTTACTTTTACGTTTTCTGTTCAAAATCAGC
TGGAT**AAGCATATCCAGCCTCACTGA**

Schematic of the genomic sequence at the target region recognized by the ZFN pair flanking the ZFN cut site and the CEL-I primer sequences.

CEL-I Primers – **Bolded and underlined**
ZFN binding site – **UPPER CASE, BOLDED RED**
ZFN cut site – lowercase red
Deletion – yellow highlighted
Insertion – green highlighted

Genotype: deletion 2/deletion 4/deletion 5/ deletion 9 & insertion 1.

C2BBe1 clone: C4

Cell Line Description

Organism*: Homo sapiens* (human)

Tissue: colorectal adenocarcinoma

Age: 72 years

Gender: Male

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
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

Parental Cell Line: ATCC® Catalog No. CRL-2102™

Note: Please see CRL-2102 product datasheet from ATCC for additional information about the origin of these cell lines. Cytogenetic information is based on initial seed stock at Sigma Life Science. Cytogenetic instability has been reported in the literature for some cell lines.

Technical Note: When using C2BBe1 parental cells as a comparator in functional efflux assays, it is crucial to use parental cells with a passage number matching the knock-out cell line being tested. Lot-specific passage number is reported on the Certificate of Analysis of each parental and knock-out cell line.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Biosafety Level: 1
This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. The parental cell line, C2BBe1, was obtained from ATCC. All animal products used in the preparation of the knockout line and maintenance of both, parental and knockout clone, have been screened negative by 9CFR for adventitious viral agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. Appropriate safety procedures are recommended to be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures have been published.[5-8]

Storage/Stability
Upon receiving a shipment of frozen cells it is important the end user gives the shipment attention without delay. To ensure 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.

Precaution: It is recommended 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 the gas phase may result in the rapid expansion of the vessel, potentially blowing off its cap with dangerous force creating flying debris.

At the time a cell line is ordered, end users should also consider the culture conditions for the new cell line and make sure the appropriate medium will be available when the cells arrive.

Medium Preparation Instructions
Complete Medium: To make the complete growth medium, add fetal bovine serum, Catalog No. F4135, to a final concentration of 10% (v/v) for maintenance or 20% (v/v) for the first two passages following initial thaw in the base medium, DMEM, Catalog No. D5671. The medium is supplemented with L-glutamine, Catalog G7513, to a final concentration of 2 mM. This medium is formulated for use with a 5% CO_2 in air.

Procedure
Information from the literature was used to establish a procedure for culturing, freezing and thawing the C2BBe1 cell line.[6]

Thawing of Frozen Cells

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 (~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 of Complete Medium [20% (v/v) FBS] and spin at ~125 × g for 5–7 minutes.
4. Resuspend cell pellet with the Complete Medium [20% (v/v) FBS] 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, prior to the addition of the vial contents, the culture vessel containing the Complete Medium [20% (v/v) FBS] be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0–7.6) and temperature (37 °C).
5. Incubate the culture at 37 °C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended for the Complete Medium.

Subculturing Procedure

Volumes used in this procedure are for a 75 cm^2 flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with EDTA solution, Catalog No. E8008.
3. Add 2.0–3.0 ml of Trypsin-EDTA solution, Catalog No.T4049, to flask and incubate at 37 °C for 10 minutes to detach the cells.
4. When cells are detached, add 6.0–8.0 mL of Complete Medium [20% (v/v) FBS] and aspirate cells by gentle pipetting.
5. Add appropriate aliquots of the cell suspension into new culture vessels.
   Subcultivation Ratio: 1:6 to 1:10 when 80-90% confluent.
6. Incubate cultures at 37 °C.
7. After two passages, cells can be switched to Complete Media containing 10% (v/v) FBS.

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.

References

1. The International Transporter Consortium (2010 White Paper), Membrane transporters in drug development. *Nature Reviews Drug Discovery*, **9**, 215-236 (2010).
2. Fleming, D.O. et al., (1995) Laboratory Safety: Principles and Practice. Second edition, ASM press, Washington, DC.
3. Hay, R.J. et al., eds. (1992), ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC.
4. Caputo, J.L., Biosafety procedures in cell culture. *J. Tissue Culture Methods*, **11**, 223-227 (1988).
5. Centers for Disease Control (1999), Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 93-8395. U.S. Dept. of Health and Human Services; 4th Edition U.S. Government Printing Office Washington D.C. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm
6. Chen, W., et al. in Cell Culture Models of Biological Barriers In-Vitro Test Systems for Drug Absorptoin and Delivery. (Lehr, C-M., ed.), Taylor & Francis, (New York, NY: 2002) pp. 143-163.

Additional product and technical information can be obtained from the catalog references and the Sigma Life Science Website (www.wherebiobegins.com/biocells).

Please see the Label License Agreement (LLA) for further details regarding the use of this product. The LLA is available on our website at www.wherebiobegins.com/biocells

These cells are distributed for research purposes only. Sigma Life Science requires that individuals contemplating commercial use of any cell line first contact us to negotiate an agreement. Third party distribution of this cell line is prohibited.

MB,ADM,PHC 08/11-2