

Product Information

CompoZr® Disease Model Cell Lines DLD-1 Cells BAX $-/-$, BAK $-/-$

Catalog Number **CLLS1005**

Storage Temperature $-196\text{ }^{\circ}\text{C}$ (liquid nitrogen)

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 deletions at both the BAX and BAK loci (see Figures 1a and 1b). Single cell knockout clones were isolated and followed for more than twenty passages to establish stable cell lines.

While the targeted genes in this cell line are diploid, 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. Modified cell lines provide the basis for the development of various assays for compound screening. Here, the target genes and corresponding protein expression are eliminated, in contrast to cell lines with normal expression.

Bcl-2 Associated X protein, commonly referred to as Bax, is a member of the Bcl-2 gene family and promotes apoptosis.¹ Its level of expression has been observed to be altered in cancer.²

BAK, Bcl-2 homologous antagonist/killer 1, another member of the Bcl-2 gene family, like BAX has also been associated with oncogenesis such as gastrointestinal cancers.³ It has been noted that induction of apoptosis by some mediators requires both BAX and BAK.⁴ Cells lacking both BAX and BAK are impaired in their ability for the intrinsic induction of apoptosis as demonstrated by cell survival when treated with the apoptotic effector staurosporine, compared to the wild-type counterpart.

Generation of this cell line lacking both BAX and BAK expression (Figure 1b) allows the assessment of activity of antitumor compounds in their absence, as well as facilitating investigation of other pathways contributing to apoptosis.⁵

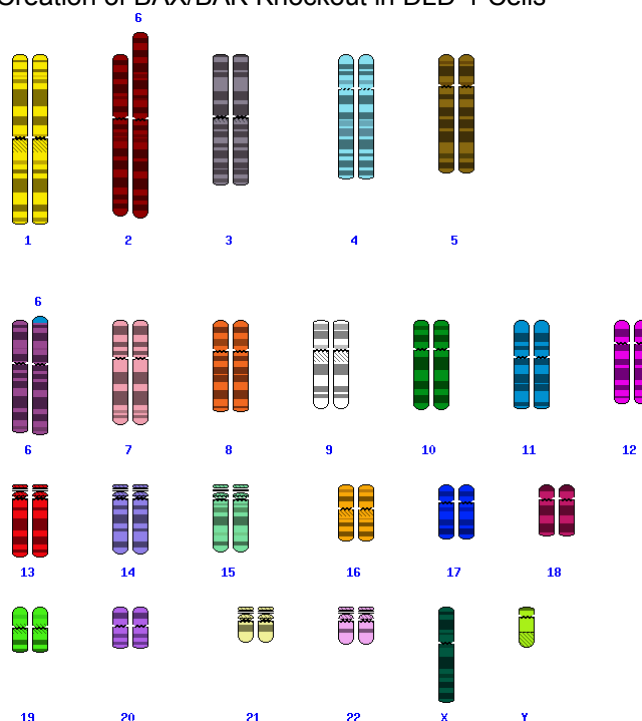
For further information and to download sequence of the modified locus, go to the website:
www.wherebiobegins.com/biocells

Components

DLD-1 mutant cell line with BAX and BAK genes knocked out 1 vial
 Catalog No. CLL1007

Parental colon adenocarcinoma cell line 1 vial
 (ATCC Catalog No. CCL-221)
 Catalog No. CLL0001

Figure 1a.
 Creation of BAX/BAK Knockout in DLD-1 Cells



BAX and BAK are diploid in DLD-1 cell line:
 locations – 19q13.3 (BAX) and 6p21.3 (BAK)
 (from NCBI SKY/M-FISH database)

Figure 1b.

Site-specific deletion at the BAX Locus (exon 5) in DLD-1 cell line

Allele 1 and 2 (homologous deletion/insertion – del 63/ins 3):

CAGTGGGGACAAGGTTTCAGTCCCTAACGCCCACT
 CCACTCCCCACAGGCC**TGTGCACCAAGGTGCCG**
GAACTGATCAgaaccaTCATGGGCTGGACATTGGAC
TTCCTCCGGGAGCAGCGGCTGTTGGGCTGGATCC
 AAGACCAGGGTGGTTGGGTGAGACTCCTCAAGCCT
 CCTCACCCCCACCACCGCGCCCTCACCACCGCCC
 CTGCCCCACCGTCCCTGCCCCCGCCACTCCTCT
 GGGACCCTGGGCCTTCTGGAGCAGGTACAGTGG
 TGCCCTCTCCCATCTTCAGATCATCAGATGTGGT
 CTATAATGCGTTTTCTTACGTGTCTGATCAATCCC
 CGATTATCTACCCTGCTGACCTCCAGTG**ACCCC**
TGACCTCACTGTGAC

Site-specific deletion at the BAX Locus (exon 1) in DLD-1 cell line

Allele 1 and 2 (homologous deletion – del 38):

ATCCTTTTTAGCCCATGCCTGCCCCTTCTCCTGGA
 GCCCAGCACTCATGTCTTAGAACACTGTCAGGGAC
 CTCCAAGAAGGGATGCACAGGGCCATGGACAGCT
 CAGGCAGAACCCTCTGCCATGAGCCAGGGCCTG
 GTCCCGACTGCCTGGTTACTGGCTCACCTGCATGC
 CTCCTG**CTCCTACAGCACCATGGGgcaggTGGGACG**
GCAGCTCGCCATCATCGGGGACGACATCAACCGA
 CGCTATGACTCAGAGTTCCAGACCATGTTGCAGCA
 CCTGCAGCCCACGGCAGAGAATGCCTATGAGT**ACT**
TCACCAAGATTGCCACC

Schematics of the genomic sequences at the target regions (exons 5 and 1, respectively) recognized by the ZFN pair and the CEL-I primer sequences:

CEL-I Primers – **Bolded and underlined**
 ZFN binding site – **UPPER CASE, BOLDED RED**
 ZFN cut site – **lower case red**
 Deletion – **yellow highlighted**
 Inserted bases - **UPPER CASE, BOLDED BLUE**

Genotype: BAX - del 63, ins 3; BAK – del 38.

DLD-1 clone C9-1

Cell Line Description

1 vial of modified DLD-1 cells contains $\sim 2 \times 10^6$ cells.

Organism: *Homo sapiens* (human)

Tissue: adenocarcinoma; colorectal

Age: adult

Gender: Male

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile

Short Tandem Repeat (STR) analysis:

Amelogenin: X,y
 CSF1PO: 11, 12
 D13S317: 8, 11
 D16S539: 12, 13
 D5S818: 13
 D7S820: 10, 12
 TH01: 7, 9.3
 TPOX: 8, 11
 vWA: 18, 19

Parental Cell Line: ATCC Catalog No. CCL-221

Note: Please see CCL-221 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.

Medium: Fetal bovine serum, Catalog No. F4135, at a final concentration of 10% (v/v) in RPMI, Catalog No. R5886, supplemented with L-glutamine, Catalog No. G7513, to a final concentration of 2 mM and sodium pyruvate, Catalog No. S8636, at 1 mM final concentration. This medium is formulated for use with a 5% CO₂ in air atmosphere.

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

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, DLD-1, 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.⁶⁻⁹

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) in the base medium, RPMI, Catalog No. R5886. The medium is supplemented with L-glutamine, Catalog No. G7513, to a final concentration of 2 mM and sodium pyruvate, Catalog No. S8636, to a final concentration of 1 mM. This medium is formulated for use with a 5% CO₂ in air atmosphere.

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.

Procedure

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 and spin at ~125 × g for 5–7 minutes.
4. Resuspend cell pellet with the Complete Medium and dispense into a 25 cm² or a 75 cm² 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 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% CO₂ in air atmosphere is recommended for the Complete Medium.

Subculturing Procedure

Volumes used in this procedure are for a 75 cm² flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.

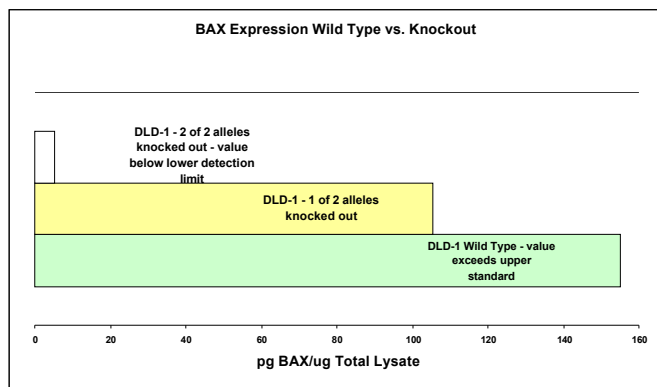
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924)
3. Add 2.0–3.0 ml of Trypsin-EDTA solution to flask and incubate at 37 °C for 10 minutes to detach the cells.
4. Add 6.0–8.0 ml of Complete Medium and aspirate cells by gentle pipetting.
5. Add appropriate aliquots of the cell suspension into new culture vessels.
Subcultivation Ratio: 1:3 to 1:6
6. Incubate cultures at 37 °C.

Note: More information on enzymatic dissociation and subculturing of cell lines is available in the literature.¹⁰

Results

Figure 2.

Loss of BAX expression



BAX expression levels were determined for the wild type DLD-1 line, a heterozygous knockout line, and the BAX homozygous knockout clone using an immunosorbent assay specific for Bax protein (Assay Designs – Enzo Life Sciences). The absolute amounts of Bax were determined by comparing values to a standard curve derived using recombinant Bax protein. The wild type lysate contained >155 pg (value exceeded value of highest standard) of Bax protein per μg of whole cell lysate compare to a calculated amount of <6 pg/ μg cell lysate (below lowest value of standard curve) for the homozygous knockout (Figure 2).

Table 1.

Loss of BAX expression

Clone	dd Ct	% Reduction
wild type	N/A	N/A
one allele disrupted	0.54	31
homozygous disruption	no Ct	100

The levels of mRNA were determined using a standard qRT-PCR assay. Briefly, 200 ng of total RNA were examined from each of three clones: wild type, one allele knocked out, and the homozygous knockout clone. The Ct values were normalized against the housekeeping gene GAPDH. The homozygous knockout did not yield a Ct (value greater than 45), compared to a calculated value of 31% loss for the heterozygous knockout (Table 1).

References

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

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