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Product Information

CompoZr® Disease Model Cell Lines
DLD-1 Cells AKT2 –/–

Catalog Number **CLLS1133**
Storage Temperature –196 °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 the AKT2 locus (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 gene in this cell line is 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 gene and resulting protein are disrupted, in contrast to cell lines with normal expression.

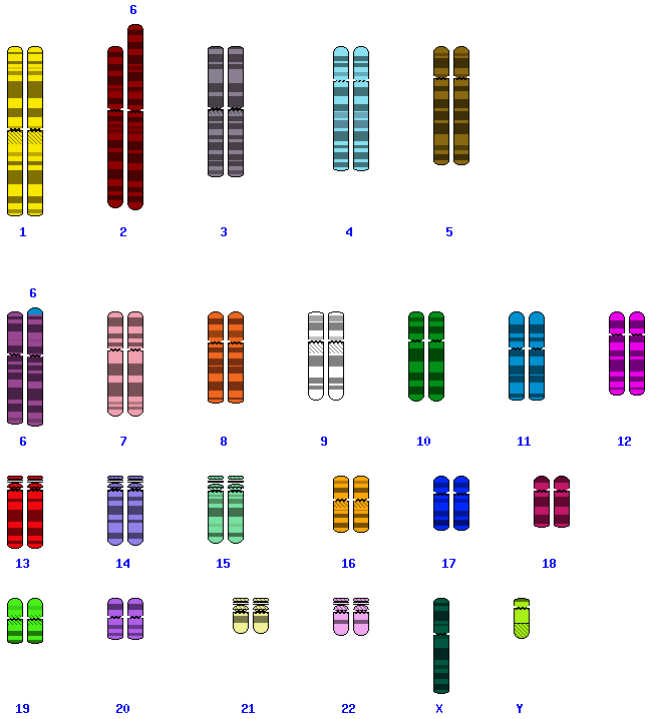
AKT2 is a serine/threonine kinase that acts downstream of phosphatidylinositol 3 kinase (PI3K) in response to growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin, and insulin-like growth factor (IGF-1).[1,2]

PI3K phosphorylates PIP_2 [phosphatidylinositol 3,4-bisphosphate, PtdIns (3,4) P_2] to form PIP_3 [phosphatidylinositol 3,4,5-triphosphate, PtdIns (3,4,5) P_3]. AKT2 contains a pleckstrin homology domain (PH) that binds PIP_3, thereby, localizing AKT2 to the membrane.[3,4] AKT is phosphorylated and activated by PDK1.[5] Once activated, AKT2 mediates antiapoptosis, cell survival, and proliferation through interactions with GSK3β, BAD, MDM2, p21, caspase 9, FOXO1A, FOXO3A, and mTOR.[6-11]

Overexpression of AKT2 transformed NIH3T3 cells and resulted in tumor formation in nude mice.[12] Over-expression of AKT2 has also been shown to play a role in enhancing cellular invasion by up-regulated β1 integrins both *in vivo* and *in vitro*, suggesting AKT2 plays a critical role in tumor metastasis.[13,14] Moreover, elevated levels of AKT2 and PI3K have been detected in primary breast cancer carcinomas and implicated in colorectal metastasis.[15]

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

Figure 1a.
Creation of AKT2 Knockout in DLD-1 Cells



AKT2 is diploid in DLD-1 cell line: location – 19q13.1-q13.2 (from NCBI SKY/M-FISH database):

Figure 1b.
Site-specific deletion at the AKT2 Locus in the DLD-1 cell line:

Allele 1 – 5 bp deletion:
TTGCCCAAA**TTCCCATGGTTATGTGGTCA**TTTGCTT
GCCTGTTGCTGGGGGTGGCTGGGAGGTGGCACTC
AGCTGTTCGACTCTGAGGCCCAGGACTCCTGGCTG
CTGGGACTCACGTGTGTCCCTGCACAGGGAGGAG
TGGATGCGGGCCATCCAGATGGTCGCCAACAGCC
TCAAGCAGCGGGCCCCAGGCGAGGACCCCATGGA
CTACAAG**TGTGGCTCCCCCAGT**gactcc**TCCACGACT**
GAGGAGATGGAAGTGGCGGTCAGCAAGGCACGG
GCTAAAGTGGTAAGTGCTGGGAGCAGGGGTGGGG
CTGCCAGCCTGCGGTGCCCCCACCCCCAGTGCTG
GCCTGCTCTCCTTGGTTTCCA**TAACACAGTTCAGG**
GGGCTCAGCTGAGAGACAAGGCTGCCCTGCAGGG
TGGGGT

Allele 2 – 11 bp deletion:
TTGCCCAAA**TTCCCATGGTTATGTGGTCA**TTTGCTT
GCCTGTTGCTGGGGGTGGCTGGGAGGTGGCACTC
AGCTGTTCGACTCTGAGGCCCAGGACTCCTGGCTG
CTGGGACTCACGTGTGTCCCTGCACAGGGAGGAG
TGGATGCGGGCCATCCAGATGGTCGCCAACAGCC
TCAAGCAGCGGGCCCCAGGCGAGGACCCCATGGA
CTACAAG**TGTGGCTCCCCCAGT**gactcc**TCCACGACT**
GAGGAGATGGAAGTGGCGGTCAGCAAGGCACGG
GCTAAAGTGGTAAGTGCTGGGAGCAGGGGTGGGG
CTGCCAGCCTGCGGTGCCCCCACCCCCAGTGCTG
GCCTGCTCTCCTTGGTTTCCA**TAACACAGTTCAGG**
GGGCTCAGCTGAGAGACAAGGCTGCCCTGCAGGG
TGGGGT

Schematic of the genomic sequence at the target region (exon 5) recognized by the ZFN pair; the resulting deletion, 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

Genotype: del 5/del 11 (heterozygous)

Components

DLD-1 mutant cell line with AKT2 gene knocked out Catalog No. CLL1133	1 vial
Parental colon adenocarcinoma cell line (ATCC® Catalog No. CCL-221™) Catalog No. CLL0001	1 vial

Cell Line Description
1 vial of modified DLD-1 cells contains ~2×10^6 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 at 1 mM final concentration (Catalog No. S8636). This medium is formulated for use with a 5% CO_2 in air.

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, A549, 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.[16-19]

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% CO_2 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 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 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 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 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.[20]

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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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