

Product Information

CompoZr® Disease Model Cell Lines DLD-1 Cells HIF1A $-/-$

Catalog Number **CLLS1002**

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 the HIF1A 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 corresponding protein expression are eliminated, in contrast to cell lines with normal expression.

Hypoxia-Inducible Factor 1-alpha (HIF1A) plays an essential role in cells responding to low oxygen or hypoxic conditions.¹ HIF1A expression appears essential to tumor angiogenesis² and overexpression has been associated with poor prognosis in colorectal cancer.^{3,4} The availability of cell lines with HIF1A expression knocked out allows the investigation of the potential roles of therapeutic agents.⁵

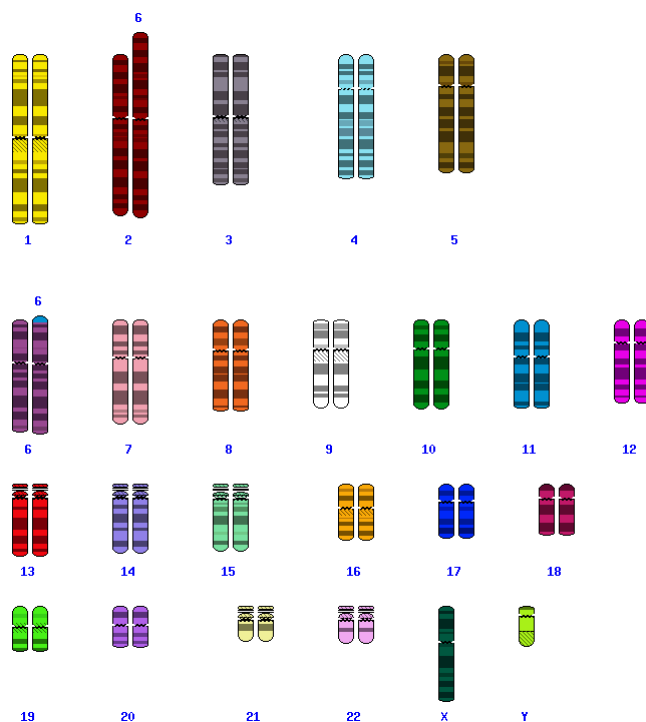
For further information and to download sequence of modified locus, go to the website:
www.wherebiobegins.com/biocells

Components

DLD-1 mutant cell line with HIF1A gene knocked out 1 vial
 Catalog No. CLL1003

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

Figure 1a.
 Creation of HIF1A Knockout in DLD-1 Cells



HIF1A is diploid in DLD-1 cell line: location – 14q21-q24 (from NCBI SKY/M-FISH database)

Figure 1b.

Site-specific deletion at the HIF1A Locus in DLD-1 cell line – Alleles 1 and 2:

AATGTCTCCATTACCCACCGCTGAAACGCCAAAGC
CACTTCGAAGTAGTGCTGACCCTGCACTCAATCAA
GAAGTTGCATTAATAAATTAGAACCAAATCCAGAGTCA
CTGGAACCTTTCTTTTACCATGCCCCAGATTCAGGAT
CAGAC**CACCTAGTCCTTccgatgGAAGCAC**TAGACAA
AGTTCACCTGAGgtaggtgtcatgatataatcagaaaggacaac
tttcagatttaacattcaagaatgtattataagtttgattcaaacactcattga
accacaaattacattgtgtgtgtttgaatttagacactttaaattattgca
agagctact**gcctaaccctagacctgagca**

Schematic of the genomic sequence at the target region (exon 10) 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**

Exon – UPPER CASE (except for cut site)

Intron – lower case

Note: 22 bp deletion occurred at ZFN binding site and caused open reading frame shift. There is a 1 bp insertion (in **lower case blue bold**) near CEL-I reverse primer in intron.

Genotype: del 22/del 22 (homozygous)

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 (See Cautionary Note regarding expansion of cells in the Procedure section)

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.⁶⁻⁸

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.

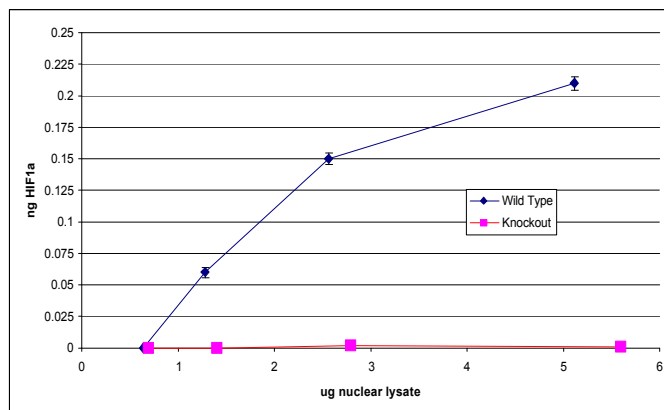
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924)
3. Add 2.0–3.0 ml of Trypsin-EDTA solution to flask and incubate at 37 °C for 10 minutes to detach the cells.
4. Add 6.0–8.0 ml of Complete Medium and aspirate cells by gentle pipetting.
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.

Cautionary Note: Due to the loss of HIF1A expression, the modified cell line is very sensitive to conditions that may induce a hypoxic response. If received/stored correctly, upon thawing, cells will demonstrate >90% viability. However, following initial attachment and growth, a fraction of the population may become non-adherent and release from the plate. Address this by changing the medium to remove non-adherent cells and maintain the culture to allow remaining cells to expand. Initial expansion of the modified cells will lag behind the wild type population. Allow the population to achieve high levels of confluence (>80%) before passage with weekly exchanges of medium. To prevent loss of cell line, early passages should include the generation of multiple stocks of frozen cells. More information on enzymatic dissociation and subculturing of cell lines is available in the literature.⁹

Results

Figure 2.

Loss of HIF1A expression



HIF1A expression was examined in wild type DLD-1 and the knockout cloned line using an enzyme-linked immunosorbent assay, ELISA (a modification of R&D Systems DYC1935-2). Briefly, both cell populations were treated 16 hours prior to harvest with medium containing reduced serum (0.1% v/v) and 0.15 mM cobalt chloride in order to mimic hypoxic conditions and induce HIF1A expression. The nuclear fraction was collected for each population and the total protein concentration determined by BCA assay (Catalog No. QPBCA). In order to determine levels of HIF1A, a standard curve was generated using recombinant HIF1A (R&D Systems, part 841691). The data represent the total amount of HIF1A (ng) detected in a sample per the total amount of nuclear lysate (μg) examined (ng of HIF1A per μg of lysate). Technical triplicates were examined for each concentration. The ELISA plates were developed using a chemiluminescent peroxidase substrate (Catalog No. CPS260).

References

1. Ratcliffe, P.J., From erythropoietin to oxygen: hypoxia-inducible factor hydroxylases and the hypoxia signal pathway. *Blood Purif.*, **20(5)**, 445-450 (2003).
2. Yang, Q.C., Inhibition of hypoxia-induced angiogenesis by trichostatin A via suppression of HIF-1 α activity in human osteosarcoma. *J. Exp. Clin. Cancer Res.*, **25(4)**, 593-599 (2006).

3. Baba, Y. et al., HIF1A overexpression is associated with poor prognosis in a cohort of 731 colorectal cancers. *Am. J. Pathol.*, **176(5)**, 2292-2301 (2010).
4. Baker, S.J. et al., Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science*, **244(4901)**, 217-221 (1989).
5. Fels, D.R. et al., Preferential cytotoxicity of bortezomib toward hypoxic tumor cells via overactivation of endoplasmic reticulum stress pathways. *Cancer Res.*, **68(22)**, 9323-9330 (2008).
6. Hay, R.J. et al., eds., *ATCC Quality Control Methods for Cell Lines*. 2nd edition, Published by ATCC (1992).
7. Caputo, J.L., Biosafety procedures in cell culture. *J. Tissue Culture Methods*, **11**, 223-227 (1988).
8. 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
9. Freshney, R.I., Chapter 10 in *Culture of Animal Cells, a manual of Basic Technique* by, 3rd edition, published by Alan R. Liss, (NY, NY: 1994).

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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