

Product Information

CompoZr® Disease Model Cell Lines MCF10A Cells HER2 -/-

Catalog Number **CLLS1045**

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 HER2 locus (see Figure 1). 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 is disrupted and the corresponding functional protein expression eliminated, in contrast to cell lines with normal expression.

HER2 (ERBB2, HER/neu, CD340), is a receptor tyrosine kinase and a member of the ErbB protein family.¹ HER2 is a recognized proto-oncogene and is located on human chromosome 17.² The HER2/neu gene product was initially determined to demonstrate homology to the EGF receptor and mapped to the neu oncogene site.³⁻⁵ Currently, 20–30% of all diagnosed breast cancers demonstrate increased expression of HER2.^{6,7} The parental MCF10A near-normal breast epithelial line has been characterized by flow cytometry and immunohistology as HER2 low/dull,^{8,9} reflecting what is considered as a “normal” level of expression. Cell lines have been derived from this parental line following prolonged culture and have shown to change expression of breast cancer-relevant targets associated with transition to the tumorigenic state.^{10,11}

By providing a near-normal breast cell line incapable of expressing fully functional HER2, studies examining tumorigenic transition and the role of exogenous agents can be conducted in the absence of its signaling. The nucleotide deletions occur in exon 27 in this cloned cell line. These nucleotide deletions correspond to deletions at the protein level affecting amino acids 1221-1255, at the carboxy terminus. The major autophosphorylation sites of HER2 are Tyr¹²⁴⁸ and Tyr^{1221/1222}. Phosphorylation of these sites couples the HER2 receptor to the Ras-Raf-MAP kinase signaling transduction pathway. Lack of functional expression of HER2 (see Figure 2) allows examination of its role in the survival pathways including components such as Ras, PI3-K, AKT, and Bcl-xl-2.

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

Components

MCF10A mutant cell line with HER2 gene knocked out 1 vial
Catalog Number CLL1045

Parental mammary epithelial cell line 1 vial
(ATCC® Catalog Number CRL-10317™)
Catalog Number CLL1040

1 vial of modified MCF10a cells contains ~2 × 10⁶ cells.

The cryoprotectant medium used is 1× Cell Freezing Medium-DMSO, Catalog Number C6164.

Figure 1.

Creation of HER2 Knockout in MCF10A Cells

Site-specific deletion at the HER2 Locus in MCF10A cell line.

Allele 1 – 124 bp deletion:

ATGGGGTCGTC**AAAGACGTTTTTGCCTTTGG**GGGT
 GCCGTGGAGAACCCCGAGTACTTGACACCCCAGG
 GAGGAGCTGCCCTCAGCCCCACCCTCCTCCTGC
 CTTACAGCCCAGCCTTCGACAACCTCTA**TACTGGG**
ACCAGGACCCACCAGAGCGGGGGGCTCCACCCAG
CACCTTCAAAGGGACACCTACGGCAGAGAACCCAG
AGTACCTGGGTCTGGACgtgcc**AGTGTGAACCAGAA**
GGCCAAGTCCGCAGAAGCCCTGATGTGCCTCAG
 GGAGCAGGGAAGGCCTGACTTCTGCTGGCATCAA
 GAGGTGGGAGGGCCCTCCGACCACTTCCAGGGGA
 ACCTGCCATGCCAGGAACCTGTCCTAAGGAACCTT
CCTTCCTGCTTGAGTTCCAGATGGCTGGAAGGG
 GTCCAGCCTCGTTGGAAGAGGAACAGCACTGGGG
 AGTCTTTGTGGATTCTGAG

Allele 2 – 25 bp deletion:

ATGGGGTCGTC**AAAGACGTTTTTGCCTTTGG**GGGT
 GCCGTGGAGAACCCCGAGTACTTGACACCCCAGG
 GAGGAGCTGCCCTCAGCCCCACCCTCCTCCTGC
 CTTACAGCCCAGCCTTCGACAACCTCTATTACTGGG
 ACCAGGACCCACCAGAGCGGGGGGCTCCACCCAG
 CACCTTCAAAGGGACACCTACGGCAGAGAACCCAG
AGTACCTGGGTCTGGACgtgcc**AGTGTGAACCAGAA**
GGCCAAGTCCGCAGAAGCCCTGATGTGCCTCAG
 GGAGCAGGGAAGGCCTGACTTCTGCTGGCATCAA
 GAGGTGGGAGGGCCCTCCGACCACTTCCAGGGGA
 ACCTGCCATGCCAGGAACCTGTCCTAAGGAACCTT
CCTTCCTGCTTGAGTTCCAGATGGCTGGAAGGG
 GTCCAGCCTCGTTGGAAGAGGAACAGCACTGGGG
 AGTCTTTGTGGATTCTGAG

Schematic of the genomic sequence at the target region (exon 27) 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 124/del 25 (heterozygous)

Cell Line DescriptionOrganism: *Homo sapiens* (human)

Tissue: mammary gland; breast

Age: 36 years

Gender: Female

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile

Short Tandem Repeat (STR) analysis:

Amelogenin: X

CSF1PO: 10,12

D13S317: 8,9

D16S539: 11,12

D5S818: 10,13

D7S820: 10,11

THO1: 8,9.3

TPOX: 9,11

vWA: 15,17

Parental Cell Line: ATCC Catalog Number CRL-10317

Note: Please see CRL-10317 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.

Complete Medium: Dulbecco's Modified Eagle's Medium (DMEM)/Ham's Nutrient Mixture F12 (1:1) with 2.5 mM L-glutamine, 5% horse serum, 10 µg/mL human insulin, 0.5 µg/mL hydrocortisone, 10 ng/mL EGF, and 100 ng/mL cholera toxin. This medium is formulated for use with a 5% CO₂ in air atmosphere.

Medium Components:

Cholera Toxin from *V. cholerae*,
 Catalog Number C8052
 DMEM/F12, Catalog Number 51448C
 Insulin Solution, Catalog Number I9278
 Epidermal Growth Factor, Catalog Number E9644
 50 µM Hydrocortisone Solution,
 Catalog Number H6909
 Horse Serum, Catalog Number H1270

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, MCF10A, 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.¹²⁻¹⁵

Preparation Instructions

Complete Medium: To make the complete growth medium combine the following:

1 L of DMEM/F12 (Catalog Number 51448C)
 50 mL of horse serum (Catalog Number H1270)
 29 mL of 50 µM Hydrocortisone Solution
 (Catalog Number H6909)
 1.08 mL of Insulin Solution (Catalog Number I9278)
 108 µL of Cholera toxin solution (1 mg/mL), prepared by dissolving Catalog Number C8052 in sterile water. Store solution at 2–8 °C.
 10.8 µL of EGF solution (1 mg/mL), prepared by dissolving Catalog Number E9644 in 10 mM acetic acid, followed by 0.2 µm filtration. Store the solution in aliquots at –20 °C.

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 $\sim 125 \times g$ 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.

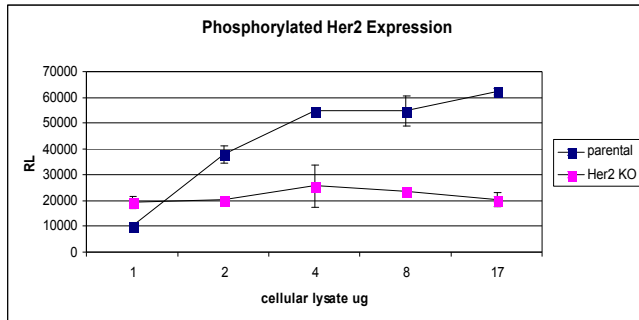
1. Cells prefer to grow in a more dense environment. Allow cells to become 90-95% confluent before attempting to pass.
2. Remove and discard culture medium.
3. Briefly rinse the cell layer with Accutase[®] (Catalog Number A6964). This cell dissociation solution does not contain mammalian or bacterial-derived products and has been observed to be gentler regarding the dissociation/detachment of this cell line.
4. Add 2.0–3.0 mL of Accutase solution to the flask and incubate at 37 °C for 3–5 minutes (examine the flask every 2 minutes in order to minimize exposure). After the first two minutes, gently agitate cells by hitting the side of the flask with palm of hand. Examine to determine if cells have released.
5. When cells are detached, add 6.0–8.0 mL of Complete Medium and aspirate cells by gentle pipetting.
6. Gently pellet the cells, remove the supernatant, and resuspend to 6–8 mL with prewarmed (37 °C) Complete Medium.
7. Add appropriate aliquots of the cell suspension into new culture vessels. Subcultivation Ratio: 1:2 (or less in order to maintain a higher cell density to promote cell growth).
8. Incubate cultures at 37 °C.

Note: MCF10A parental cells require longer time for digestion/cell release than what is typical. However, it is recommended when passing cells to check every 5 minutes in order to minimize exposure time to Accutase. More information on enzymatic dissociation and subculturing of cell lines is available in the literature.¹⁶

Results

Figure 2.

Loss of functional HER2 expression



HER2 expression, phosphorylated, was examined in wild type MCF10A and the knockout cloned line using an enzyme-linked immunosorbent assay (a modification of R&D Systems DYC1768). Briefly, both cell populations were grown to near confluency in T75 flasks prior to harvesting as outlined in the "Subculturing Procedure". Cytoplasmic cellular lysates were prepared as outlined in the procedure (Pierce, NE-PER 78833). Total protein concentration was determined by BCA assay (Catalog Number QPBCA). Technical triplicates were examined for each concentration. The ELISA plates were developed using a chemiluminescent peroxidase substrate (Catalog Number CPS2120).

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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