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Product Information

CompoZr® Disease Model Cell Lines
MCF10A Cells HER2 –/– exon 2

Catalog Number **CLLS1214**
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.

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 targeted gene is disrupted through frame shift, and the corresponding functional protein expression was eliminated, in contrast to cell lines with normal expression.

HER2/neu, also known as ErbB-2 (Human Epidermal Growth Factor Receptor 2), is a member of the ErbB protein family. HER2 is a cell membrane receptor tyrosine kinase and is involved in the signal transduction pathway leading to cell growth and differentiation.[1,2] The HER2 gene is a proto-oncogene located at the long arm of human chromosome 17. Breast cancers are closely associated with an amplification of the HER2/neu gene or overexpression of its protein product.[3,4] Overexpression also occurs in other cancers such as ovarian cancer, stomach cancer, and biologically aggressive forms of uterine cancer.[3] HER2 is thought to be an orphan receptor, with none of the EGF family of ligands able to activate it.[5] However, ErbB receptors dimerize on ligand binding and HER2 is the preferential dimerization partner of other members of the ErbB family.[4]

HER2 ZFNs targeted at the 5th exon (2nd coding exon) of HER2 gene were transfected into the MCF10A cells. HER2 null cells were sorted out through antibody (anti-HER2/neu-PE) staining. A single cell clone with the double allele HER2 knockout (Figure 1) was confirmed by flow analysis (Figure 2) and microscopic examination (Figure 3) using anti-HER2/neu-PE immunostaining.

Components

MCF10A mutant cell line with HER2 knockout –/– 1 vial
Catalog No. CLL1214

Parental mammary epithelial cell line 1 vial
(ATCC® Catalog No. CRL-10317™)
Catalog No. CLL1040

1 vial of MCF10A cells contains ~2×10^6 cells.

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

Figure 1.
Creation of HER2 –/– in MCF10A Cells

Site-specific insertion of CACC at both alleles of HER2 Locus in the MCF10A cell line

Wild Type sequences at exon 5

GGGTCCTTCAGTCAGGCTTCTCCCTGTCTGAGGTG
GCATGACTTGGAGTGAGTTTGGATGGGGTGGCCA
GGTCTGAGAAGGTCCCCCGCCAGTGTCCTCTGAC
CCATCTGCT**CTCTCCTGCCAGTGT**gcaccg**GCACAG**
ACATGAAGCTGCGGCTCCCTGCCAGTCCCGAGAC
CCACCTGGACATGCTCCGCCACCTCTACCAGGGCT
GCCAGGTGGTGCAGGGAAACCTGGA**ACTCACCTA**
CCTGCCCACCA

HER2 –/– sequences at exon 5

Alleles 1 & 2 – 4 bp insertion

GGGTCCTTCAGTCAGGCTTCTCCCTGTCTGAGGTG
GCATGACTTGGAGTGAGTTTGGATGGGGTGGCCA
GGTCTGAGAAGGTCCCCCGCCAGTGTCCTCTGAC
CCATCTGCT**CTCTCCTGCCAGTGT**gcacc**CACC**gGC
ACAGACATGAAGCTGCGGCTCCCTGCCAGTCCCG
AGACCCACCTGGACATGCTCCGCCACCTCTACCAG
GGCTGCCAGGTGGTGCAGGGAAACCTGGA**ACTCA**
CCTACCTGCCCACCA

Schematic of the genomic sequence at the target region (exon 5) recognized by the ZFN pair, the resulting nucleotide change, 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
Insertion **- UPPER CASE, BOLDED BLUE**
Codon change - blue highlighted

Genotype: ins 4/ins 4 (homozygous) in exon 5 (causing frame shift afterwards)

Figure 2.
Flow analysis of HER2 –/– cells versus wild type (WT) MCF10A cells stained with Anti-HER2/nue-PE antibody

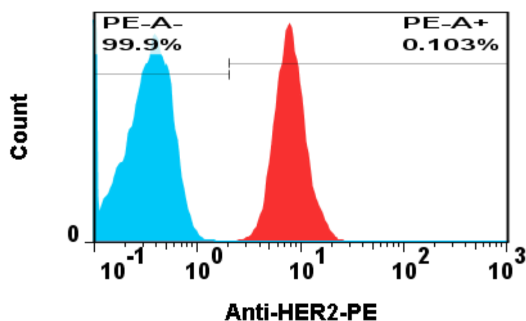
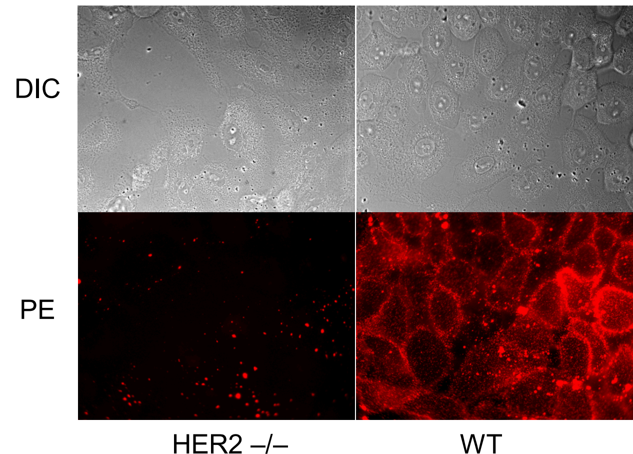


Figure 3.
Microscopic comparison of HER2 –/– cells versus wild type (WT) MCF10A cells stained with Anti-HER2/nue-PE antibody



Cell Line Description
Organism*: 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 No. 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.

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.[6-8]

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.

Preparation Instructions
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% CO_2 in air atmosphere.

Medium Components:
DMEM/F12, Catalog No. 51448C
Cholera Toxin from *Vibrio cholerae*, Catalog No. C8052
Epidermal Growth Factor, Catalog No. E9644
Horse Serum, Catalog No. H1270
Hydrocortisone Solution, 50 μM. Catalog No. H6909
Insulin Solution, Catalog No. I9278

To make the complete growth medium combine the following:

1. 1 liter of DMEM/F12
2. 108 μL of Cholera toxin solution (1 mg/mL), prepared by dissolving Catalog Number C8052 in sterile water. Store solution at 2–8 °C.
3. 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.
4. 50 mL of horse serum
5. 29 mL of Hydrocortisone Solution, 50 μM
6. 1.08 mL of Insulin Solution

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 culture flask. It is recommended to begin the culture in a 25 cm^2 flask as the initial adaptation and expansion of the cells is more rapid in a denser environment. 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. Cells prefer to grow in a denser 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 No. 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 side of 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 6.0–8.0 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.[8]

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

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