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Product Information

SKOV3 GFP-HER2
Ovarian Cell Line with GFP-HER2

Catalog Number **CLL1135**
Storage Temperature –196 °C (liquid nitrogen)

TECHNICAL BULLETIN

Product Description
This product is a human SKOV3 cell line in which the genomic HER2 gene has been endogenously tagged with a Green Fluorescent Protein (GFP) gene using CompoZr® Zinc Finger Nuclease (ZFN) technology.

CompoZr 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 homologous recombination pathway was used to insert a transgene into a desired target location – at the C terminus of the HER2 locus. A donor construct containing a fluorescent reporter gene (GFP) flanked by sequences homologous to the target site was nucleofected into SKOV3 cells along with ZFNs designed to cut near the genomic target site (see Figures 1a and 1b). Integration resulted in endogenous expression of a fluorescent fusion protein HER2-GFP. Knock-in cells were sorted to single cells by flow cytometry and expanded into clonal populations. Testing of these clones was used to select a single HER2-GFP clone as a stable cell line (Figure 2a and 2b). Junction PCR showed that at least one allele is tagged (Figure 3a) and Southern analysis showed that there were no off-target insertions of the GFP (Figure 3b).

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]

ZFN-mediated gene tagging in knock-in cell lines provides a basis for the development of various assays for compound screening. Unlike cell lines with overexpression of the gene driven by an exogenous promoter, target gene regulation and corresponding protein function are preserved in this cell line (see Figures 2a and 2b).

GFP and TagGFP2 are all synonymous for the fluorescent reporter gene in this document. The GFP used in this cell line originated from Evrogen, referred to as TagGFP2:
http://evrogen.com/products/TagFPs.shtml

For further information on our CompoZr modified cell lines go to the website:
www.wherebiobegins.com/biocells

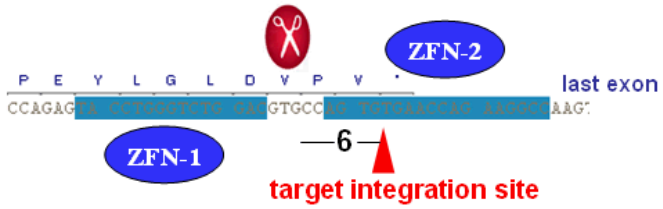
Component

SKOV3 knock-in cell line with HER2 gene C-terminally tagged with GFP Catalog No. CLL1135	1 vial

1 vial contains ~2×10^6 cells in Cell Freezing Medium-DMSO 1×, Catalog No. C6164.

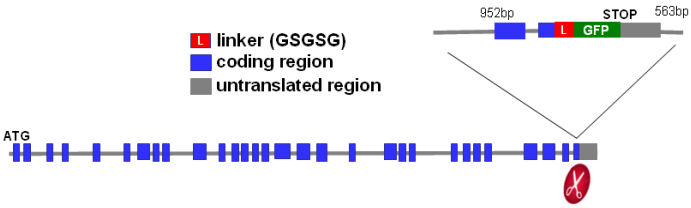
The design of tag sequence integration at the HER2 locus

Figure 1a.



Schematic of the genomic sequence of HER2's last exon at the target region for integration of the fluorescent tag GFP. CompoZr ZFN binding sites (blue boxes), the ZFN cut site (scissors), and the tag sequence integration site (red arrow).

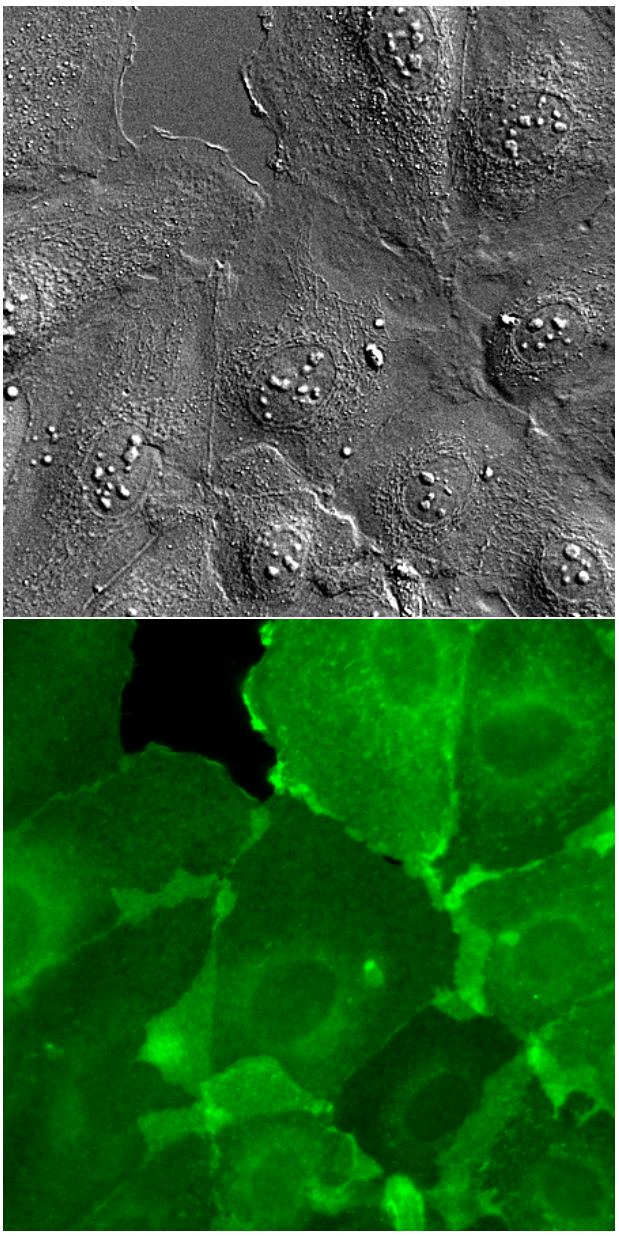
Figure 1b.



Schematic of HER2 locus showing the coding region (blue), untranslated region (gray), and the ZFN cut site (scissors). The donor (top) has the homology arms of indicated length and the GFP sequence (green) fused at the end of HER2 coding sequence (a C-terminal fusion).

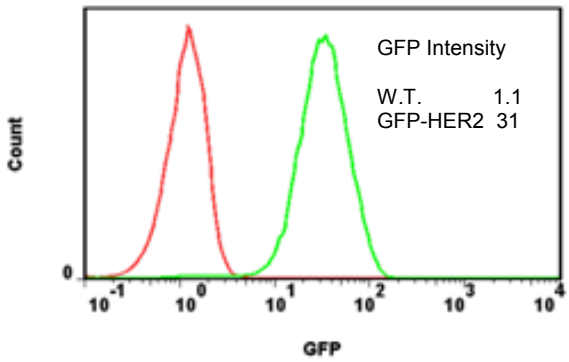
Localization & expression levels of endogenously-tagged HER2

Figure 2a.



Differential interference contrast (DIC) and fluorescence microscopy images of an isolated cell clone expressing endogenous HER2 protein tagged with GFP (ex 450–490/em 500–550, 40×/1.4 oil/1 second exposure).

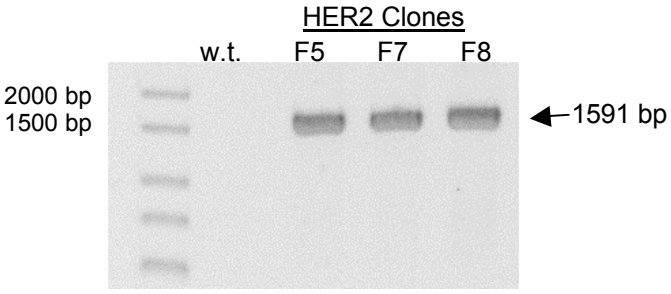
Figure 2b.



Fluorescence analysis of the GFP-HER2 clone compared to wild type SKOV3 (auto fluorescence) using MACS Quant from Miltenyi Biotec.

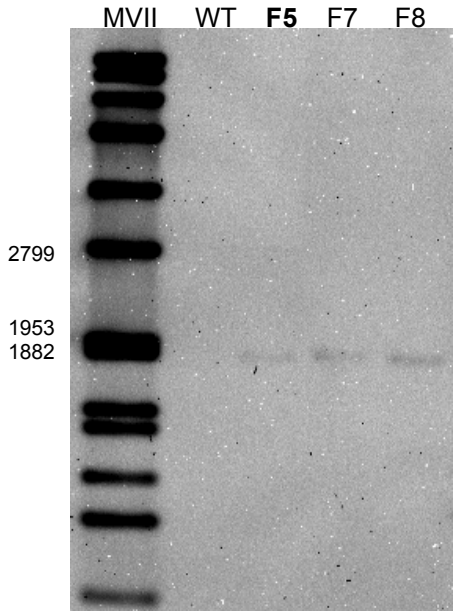
Molecular diagnostics of endogenously tagged HER2 clones

Figure 3a.



Junction PCR confirms the targeted integration (TI) of GFP at the site of the HER2 locus. PCR was performed on genomic DNA isolated from wild type and HER2 tagged clones F5, F7, and F8. The sense primer binds to HER2 sequence outside homology arm (5'-agttggtgtctgaattctcccgca) and the anti-sense primer binds to GFP sequence (5'- acttgatctccagcttgccgtagt).

Figure 3b.



Southern blotting shows no random integration from the plasmid donor initially used to create the clones. Genomic DNA from single cell clones F5, F7, and F8 were digested with KpnI. Accurate targeted insertion of the GFP into the HER2 locus should produce a hybridized band of 1868 bp in size. Dig-labeled GFP probe was used. HER2 clone F5 (bolded) was chosen as the final product.

Cell Line Description
Organism*: Homo sapiens* (human)

Tissue: ovary

Age: 64 years

Gender: Female

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
Amelogenin: X
CSF1PO: 11
D13S317: 8, 11
D16S539: 12
D5S818: 11
D7S820: 13,14
TH01: 9,9.3
TPOX: 8,11
vWA: 17,18

Parental Cell Line: ATCC® Catalog No. HTB-77™

Note: Please see HTB-77 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, SKOV3, 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.

Complete Medium Preparation Instructions
To make the complete growth medium, Add L-Glutamine, Catalog No. G7513, at a final concentration of 1.5 mM, and fetal bovine serum, Catalog No. F2442, at a final concentration of 10% in the base medium, McCoy's 5A medium, Catalog No. M8403. This medium is formulated for use with a 5% CO_2 in air atmosphere.

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.

Sub-culturing 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 5 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.
   Sub-cultivation Ratio: 1:3
6. Incubate cultures at 37 °C.

Note: More information on enzymatic dissociation and subculturing of cell lines is available in the literature.[8]

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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