

## Product Information

### U2OS GFP-NUP98

#### Osteosarcoma Cell Line with 3XFLAG™-GFP-tagged NUP98

Catalog Number **CLL1136**

Storage Temperature –196 °C (liquid nitrogen)

## TECHNICAL BULLETIN

### Product Description

This product is a human U2OS cell line in which the genomic NUP98 gene has been endogenously tagged with a Green Fluorescent Protein (GFP) gene using CompoZr® Zinc Finger Nuclease (ZFN) technology. The cell line is useful for studying the subcellular localization of NUP98 as it relates to regulating nuclear processes.

CompoZr 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.compozrzn.com](http://www.compozrzn.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 the desired target location – behind the start codon of the NUP98 locus, which tags all the variants of NUP98. (NUP98 variant 1 is NM\_016320). A donor construct containing the epitope 3XFLAG and a fluorescent reporter gene (GFP) flanked by sequences homologous to the target site was nucleofected into U2OS cells along with ZFNs designed to cut near the genomic target site (see Figures 1a and 1b). Integration resulted in endogenous expression of fluorescent fusion protein 3XFLAG-GFP-NUP98 that plays critical roles in the nuclear pore complex (NPC) within the nuclear envelope.<sup>1-5</sup> Single cell knock-in clones were isolated and followed for more than twenty passages to establish a stable cell line expressing 3XFLAG-GFP-NUP98 from the endogenous genomic locus. PCR showed that at least one allele is tagged and at least one wild type allele remains untagged (see Figure 2a and 2b) and Southern analysis showed that there were no off-target insertions of the GFP (see Figure 2c).

NUP98 is a peripheral nucleoporin component of the NPC and recently has been shown to interact with chromatin in the nucleoplasm (away from the nuclear envelope) suggesting a dynamic and mobile regulatory role.<sup>1,2</sup> NUP98 is involved in nuclear envelope permeability<sup>3</sup>, nuclear pore complex assembly/

disassembly during cell division<sup>3,4</sup>, mitotic spindle assembly<sup>4</sup>, chromatin organization<sup>5</sup> and thus gene regulation<sup>1,2</sup>. It is not surprising that malfunctioning NUP98 is a cause of specific cancers. Some patients with acute myeloid leukemia (AML) have chromosomal translocations creating gene fusions with NUP98 and homeodomain transcription factors. The molecular understanding of this oncogenesis is that the NUP98 chimera interacts with histone acetyltransferases and alters their activities.<sup>5</sup>

ZFN mediated gene tagging in knock-in cell lines provides the basis for the development of various assays for compound screening. Specifically, the target's gene regulation and corresponding protein function are preserved in contrast to cell lines with overexpression via an exogenous promoter (see Figures 3a and 3b). Overexpression of NUP98 is believed to cause improper localization of the protein within the cell.

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](http://www.wherebiobegins.com/biocells)

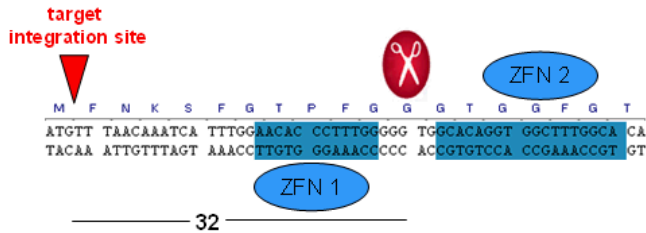
### Component

U2OS knock-in cell line having the NUP98 gene tagged at the N-terminus with 3XFLAG-GFP. 1 vial  
Catalog No. CLL1136

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

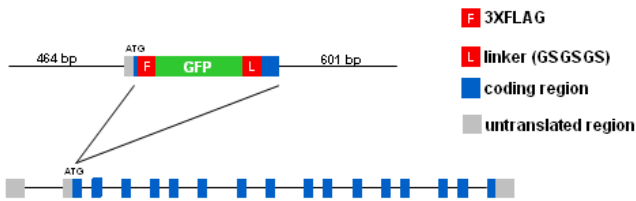
## The design of tag sequence integration at the NUP98 Locus

Figure 1a.



Schematic of the genomic sequence at the target region for integration of the epitope-fluorescent tag 3XFLAG-GFP. DNA of NUP98's exon 2, showing the start codon, CompoZr ZFN binding sites (blue boxes), the ZFN cut site (scissors), and the tag sequence integration site (red arrow).

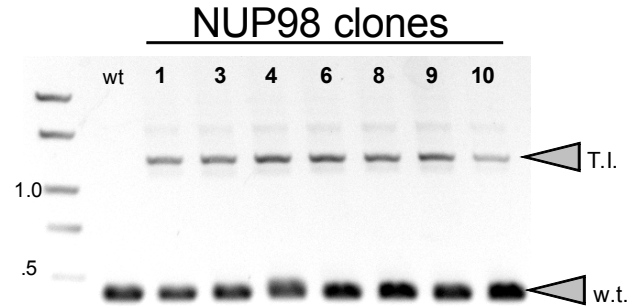
Figure 1b.



Schematic of the NUP98 locus and the donor with the locus showing the coding regions (blue) and untranslated regions (gray). The donor (top) has the homology arms of indicated length and the 3XFLAG-GFP sequence (red/green/red) fused to the beginning of the NUP98 coding sequence (an N-terminal fusion).

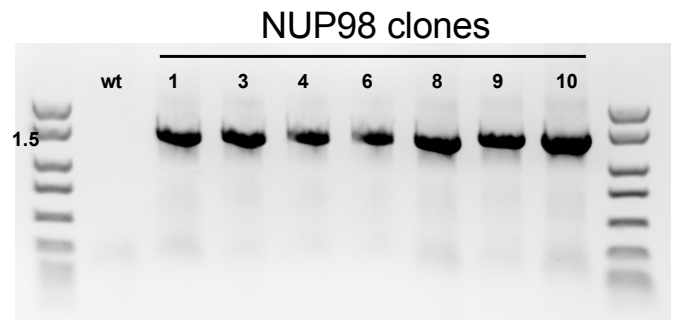
## Molecular diagnostics of endogenously-tagged NUP98 clones

Figure 2a.



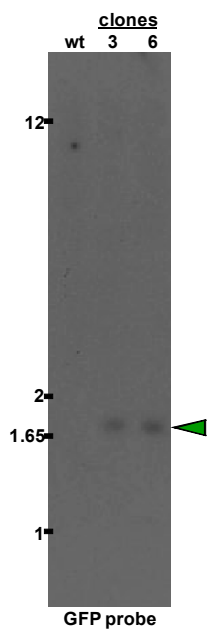
PCR using primers flanking the targeted integration (T.I.) site of the NUP98 locus shows the loci with and without GFP integration. PCR was performed on genomic DNA isolated from wild type and NUP98 tagged clones. The lower migrating band corresponds to NUP98 locus unmodified. The higher migrating band corresponds to the NUP98 locus with GFP modification.

Figure 2b.



Junction PCR confirms the higher migrating band shown is a result of GFP integration. The sense primer binds to NUP98 locus but is not contained within the NUP98 donor plasmid (5'-gagatagaagggcgggcatgcat) and the anti-sense primer binds to GFP (5'-tgtacacgtgtggctgtgaagc).

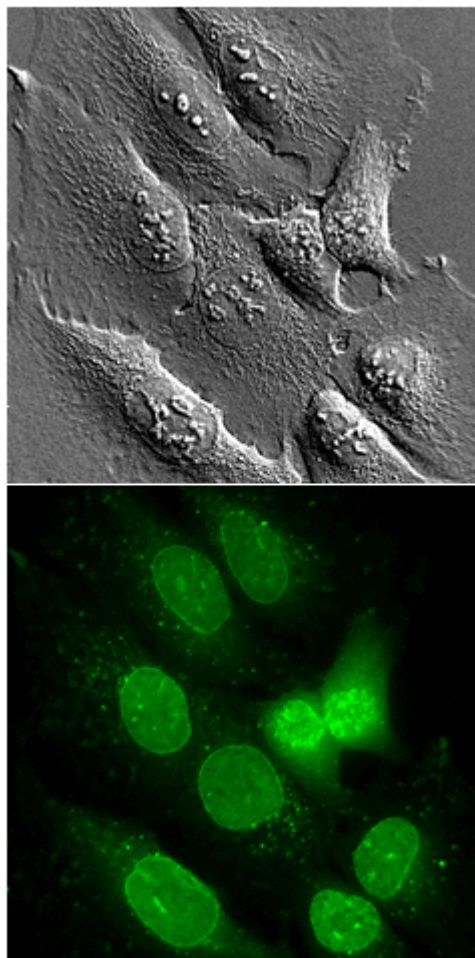
Figure 2c.



Southern blotting shows no random integration from the plasmid donor initially used to create the clones. Genomic DNA from single cell clones 3 and 6 were digested with BamHI. Proper targeted insertion of the GFP into the NUP98 locus should produce a hybridized band of approximate 1.7 Kb in size. Radioactive-labeled GFP probe was used. NUP98 clone 3 was chosen as the final product.

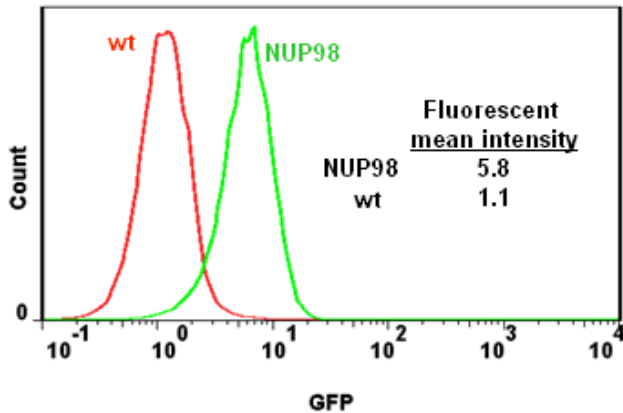
Localization &amp; expression levels of endogenously tagged NUP98

Figure 3a.



Differential interference contrast (DIC) and fluorescence microscopy images of an isolated cell clone expressing endogenous NUP98 protein tagged with 3XFLAG-GFP (ex 450–490/em 500–550, 40×/1.4 oil/1 second exposure). NUP98 endogenous expression levels are low and near autofluorescence levels (see Figure 3b).

Figure 3b.



Fluorescence analysis of the 3XFLAG-GFP-NUP98 clone compared to wild type U2OS (autofluorescence) using MACS Quant from Miltenyi Biotec.

#### Cell Line Description

Organism: *Homo sapiens* (human)

Tissue: osteosarcoma; bone

Age: 15 years

Gender: Female

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile

Short Tandem Repeat (STR) analysis:

Amelogenin: X

CSF1PO: 13

D13S317: 13

D16S539: 11,12

D5S818: 11

D7S820: 11,12

TH01: 6,9.3

TPOX: 11,12

vWA: 14,18

Parental Cell Line: ATCC® Catalog No. HTB-96™

Note: Please see HTB-96 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, U2OS, 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.<sup>6-9</sup>

#### **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  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  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 fetal bovine serum, Catalog No. F2442, to a final concentration of 10% in the base medium, McCoy's 5A Medium Modified, Catalog No. M9309. This medium is formulated for use with a 5% CO<sub>2</sub> 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<sup>2</sup> or a 75 cm<sup>2</sup> 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<sub>2</sub> in air atmosphere is recommended for the Complete Medium.

### Sub-culturing Procedure

Volumes used in this procedure are for a 75 cm<sup>2</sup> 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 7 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.<sup>10</sup>

## 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](http://www.wherebiobegins.com/biocells)).

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