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Product Information

SKOV3 GFP-STAT3
Ovarian Adenocarcinoma Cell Line with GFP-tagged STAT3

Catalog Number **CLL1139**
Storage Temperature –196 °C (liquid nitrogen)

TECHNICAL BULLETIN

Product Description

This product is a human SKOV3 cell line in which the genomic STAT3 gene has been endogenously tagged with a Green Fluorescent Protein (GFP) gene using CompoZr® zinc finger nuclease (ZFN) technology. The cell line shows redistribution of STAT3 from the cytoplasm to the nucleus upon activation with a ligand such as IL-6, making it useful for high content screening of compounds that modulate STAT3 activity.

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.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 – behind the start codon of the STAT3 locus. A donor construct containing a fluorescent reporter gene (GFP) flanked by sequences homologous to the regions on either side of the genomic target site was nucleofected into the SKOV3 cells along with ZFNs designed to cut near the target site (see Figures 1a and 1b). Integration resulted in endogenous expression of the fluorescent fusion protein GFP-STAT3. Knockin cells were sorted to single cells by flow cytometry and expanded into clonal populations. Testing of these clones was used to select a single GFP-STAT3 clone as a stable cell line (see Figures 2a and 2b). Junction PCR showed that at least one allele is tagged (see Figure 3a) and Southern analysis confirmed that there were no off-target insertions of the GFP (see Figure 3b).

Signal transducer and activator of transcription 3 (STAT3) belongs to the STAT protein family (STAT1-7) which are signaling intermediates that mediate the action of many cytokines and growth factors.[1] STAT3 is an oncogene that is constitutively active in many different cancers including prostate, breast, lung, head and neck, colon, liver, and pancreas as well as multiple myeloma and large granular lymphocytic leukemia.[2]

Numerous studies have reproducibly demonstrated inhibiting STAT3 results in decreased tumor growth and improved animal survival by inducing tumor cell apoptosis, inhibiting angiogenesis, and enhancing anti-tumor immune-mediated cytotoxicity. It has also been shown recently that a STAT3-mediated mechanism drives self-renewal and tumorigenesis of $CD24^+$ hepatic tumor-initiating cells.[3] Thus, STAT3 is recognized as a promising drug discovery target for many cancers.

To date, a few non-peptidic small-molecules have been reported to inhibit STAT3 by direct binding to its SH2 domain. Most groups used virtual screening to identify candidate compounds with an increased likelihood of binding to the STAT3 SH2 domain. The result of these studies was the identification of a specific STAT3 inhibitor called Stattic (STAT three inhibitory compound, Catalog No. S7947). Stattic selectively inhibits activation, dimerization, and nuclear translocation of STAT3, and induces apoptosis in STAT3-dependent cancer cell lines.[4]

Screening for potential STAT3 inhibitors is based on their ability to compete with a high-affinity phospho-peptide targeted to the SH2 domain of STAT3.[4] This *in vitro* assay yields many false leads because it doesn't take into account that many small molecules are not permeable to cell membranes in cell-based assays or in animal models. Therefore, a secondary screen for visualizing the inhibition of IL-6 driven STAT3 nuclear localization is utilized to identify the best compounds.[4,5] Using immunofluorescence to detect native protein localization[4] is expensive and fixation can cause reproducibility concerns in these assays. Alternatively, overexpressing a fluorescently-tagged STAT3 creates aberrant physiological conditions and may create artifactual results.[5]

With ZFN-mediated gene tagging in a knockin cell line, STAT3's native gene regulation is conserved resulting in normal protein expression levels and preservation of protein function. The endogenous STAT3 locus has been successfully tagged with either GFP or RFP sequences in SKOV3 and A549 cell lines, respectively (Catalog No. CLL1140 for A549 RFP-STAT3).

For SKOV3 GFP-STAT3, it has been confirmed fluorescently-tagged STAT3 is predominantly in the cytoplasm of uninduced cells and nuclear translocation can be detected as early as 3 minutes after the induction with 100 ng/mL of IL-6. Within 20 minutes of IL-6 treatment, GFP-STAT3 was primarily localized in the nucleus of the SKOV3 cells (see Figure 2a and Figure 4, Panels A, B). However, after preincubation of the cells with 20 μM Stattic for one hour, the IL-6 induced nuclear translocation of STAT3 was inhibited (see Figure 4, Panels C, D).

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

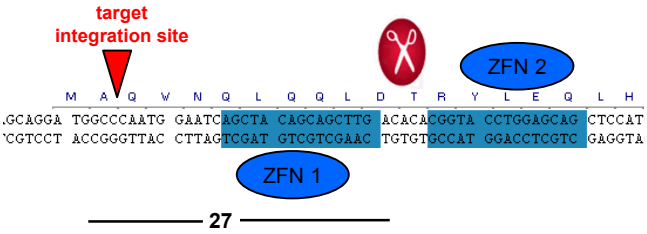
Component

SKOV3 knock-in cell line having the STAT3 gene tagged at the N-terminus with GFP Catalog No. CLL1139	1 vial

One vial of modified SKOV3 cells contains ~2×10^6 cells in Cell Freezing Medium-DMSO 1×, Catalog No. C6164.

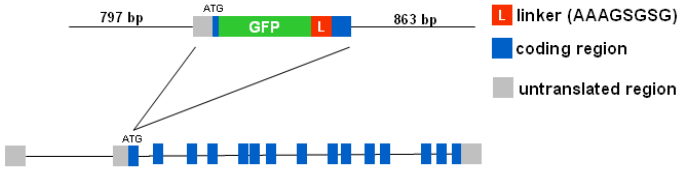
Design of Tag Sequence Integration at the STAT3 Gene Locus

Figure 1a.



Schematic of the genomic sequence at the target region for integration of the fluorescent tag GFP. DNA of STAT3, showing the start codon, CompoZr ZFN binding sites (blue boxes), the ZFN cut site (scissors), and the tag sequence integration site (red arrow).

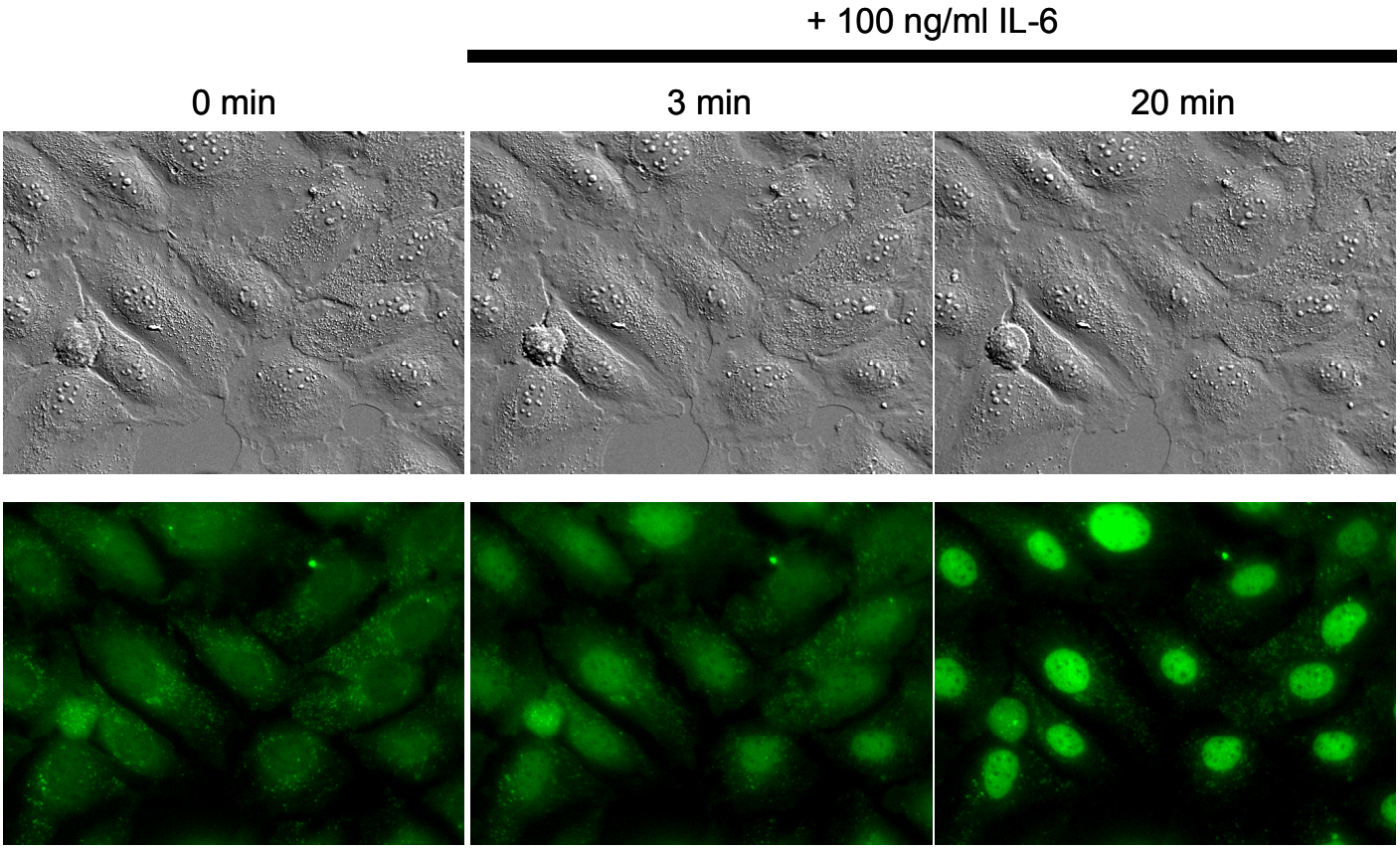
Figure 1b.



Schematic of the STAT3 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 GFP sequence (green) fused to the beginning of the STAT3 coding sequence (an N-terminal fusion).

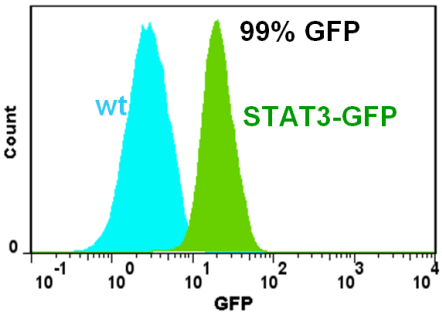
Localization and expression levels of endogenously GFP-tagged STAT3 (GFP-STAT3) in SKOV3 cells

Figure 2a.



Differential interference contrast (DIC) and fluorescence microscopy images of an isolated cell clone expressing endogenous STAT3 protein tagged with GFP without and with addition of 100 ng/mL of IL-6 (ex 450–490/ em 500–550, 40×/1.3 oil). The cells were imaged in Hanks balanced salt solution (Catalog No. H8264) supplemented with 2% fetal bovine serum (Catalog No. F2442). Endogenous STAT3 expression levels are low and near autofluorescence levels (Figure 2b) and following IL-6 stimulation nuclear translocation of GFP-STAT3 fusion protein is distinctly detectable.

Figure 2b.

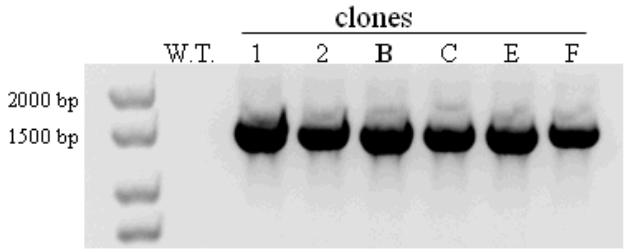


	Fluorescent Mean Intensity
SKOV3 (wt)	2.75
GFP-STAT3 in SKOV3	20.40

Fluorescence analysis of the GFP-STAT3 clone compared to wild type SKOV3 (autofluorescence) using MACSQuant®.

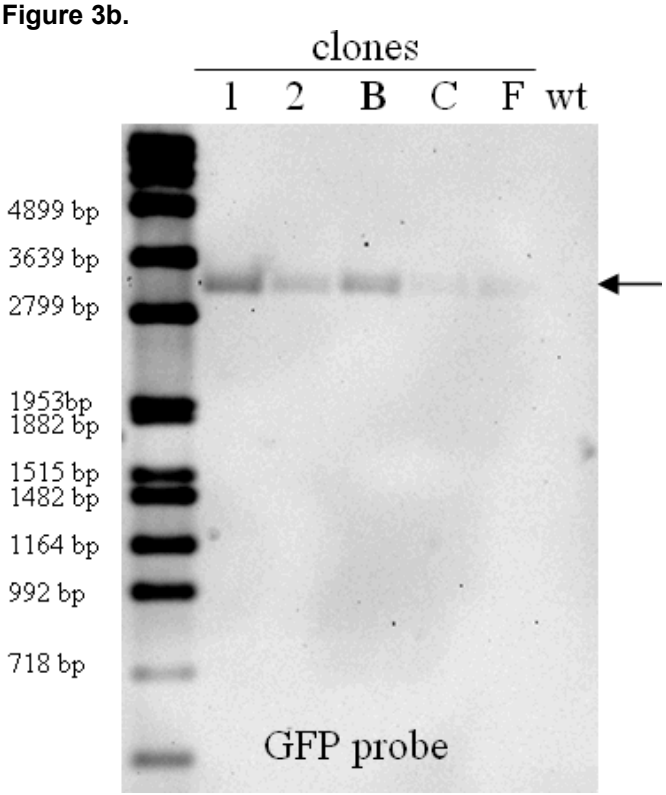
Molecular Analysis to Identify Targeted Integration in SKOV3 GFP-STAT3 Clones

Figure 3a.



Junction PCR using GFP forward and STAT3 reverse primers produced a characteristic fragment (1500 bp) for targeted integration for all clones tested. No PCR product can be detected in the SKOV3 wild type cell line.

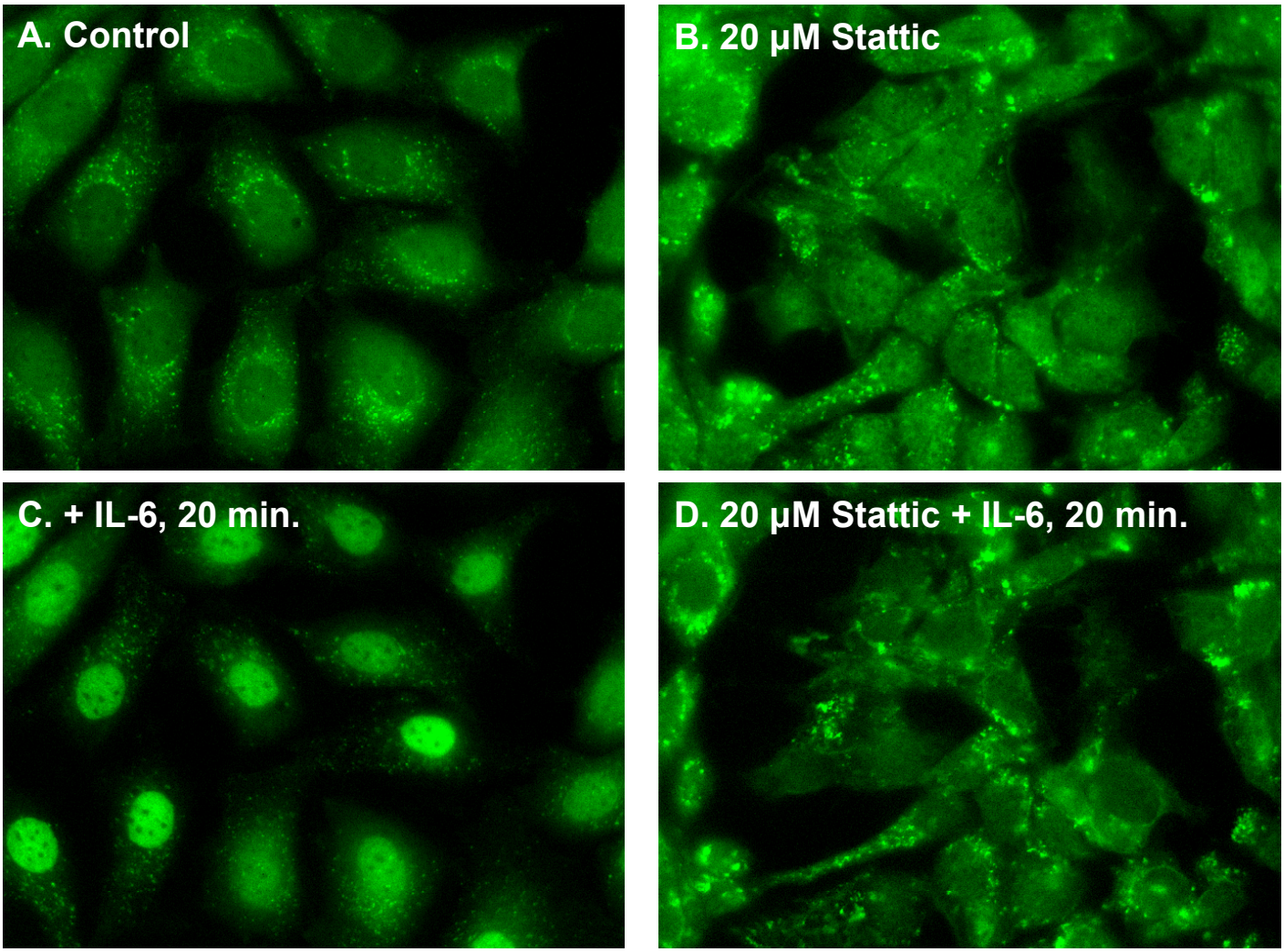
Figure 3b.



Southern blotting was performed using DIG-labeled GFP probe for single clones and genomic DNA digested with *Xba* I, *Sph* I. Only the expected DNA-hybridization band at 3300 bp can be detected across the clones. No random DNA bands were observed. Digested SKOV3 wild type served as a negative control for GFP-STAT3 integration. Clone B was selected based on cell morphology, molecular analyses, and imaging/ translocation analyses.

Inhibition of translocation of GFP-STAT3 fusion protein from the cytosol to the nucleus in SKOV3 cells by Stattic

Figure 4.



Fluorescence microscopy analysis indicated GFP-STAT3 protein was translocated from the cytoplasm (A) to the nucleus (C) when cells were cultured in medium containing 100 ng/ml of IL-6 for 30 minutes. If cells were preincubated with 20 μM Stattic for 1 hour (B, D), the IL-6 treatment did not induce the GFP-STAT3 translocation (D).

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 complete growth medium, add fetal bovine serum, Catalog No. F2442, to a final concentration of 10% and L-Glutamine, Catalog No. G7513, to a final concentration of 1.5 mM 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.

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. 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 1:20
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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