Description

ASGR1 Knockout HepG2 Cell Line is a HepG2 liver cancer cell line in which ASGR1 (Asialoglycoprotein Receptor 1), the functional subunit of the asialoglycoprotein receptor (ASGPR) complex, is no longer expressed due to CRISPR/Cas9 genome editing. This cell line was generated by electroporation of HepG2 cells with ribonucleoprotein complexes of Cas9 with an sgRNA targeting ASGR1.

This cell line has been validated by genomic sequencing, flow cytometry and an internalization assay.

Background

The asialoglycoprotein receptor (ASGPR) complex is primarily expressed on the surface of hepatocytes, and is composed of two subunits, asialoglycoprotein receptor 1 and 2 (ASGR1 and ASGR2). ASGPR binds glycoproteins with a terminal galactose (Gal) or N-acetylgalactosamine (GalNAc), directing them to endocytosis and degradation in the lysosome. ASGR1 is the functional subunit of the ASGPR complex that binds to ligands and mediates endocytosis. Because of its liver localization, endocytosis activity, and efficient recycling back to the plasma membrane, ASGR1 has been used for liver-targeted drug delivery. Givosiran (Givlaari), a treatment for a genetic liver disease, was the first GalNAc-conjugated siRNA to be approved by the FDA. Several other GalNAc-conjugated siRNAs have since been approved or are undergoing clinical trials for the treatment of hereditary liver diseases, hepatitis B virus, or metabolic disorders. For example, Inclisiran (Leqvio) targets PCSK9 (Proprotein convertase subtilisin/kexin type 9) and is under investigation for the treatment of hypercholesterolemia. ASGR1 deficiency itself is associated with increased cholesterol efflux, decreased lipid levels in blood plasma and the liver, and decreased susceptibility to cardiovascular disease, suggesting that ASGR1 may be itself a promising therapeutic target. Finally, ASGR1 is being explored as a receptor for lysosome-targeting chimeras (LYTACs), which use GalNAc to direct extracellular or membrane-bound proteins to ASGR1 for endocytosis and degradation in the lysosome.

Applications

- Useful as control when testing ASGR1-targeting drug delivery molecules, such as GalNAc-conjugated siRNAs, antisense oligonucleotides, or LYTACs.
- Useful for testing ASGR1 inhibitors.
- Useful for studying phenotypes related to ASGR1 deficiency in human liver cells.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HepG2, human hepatoma cell line, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. Cells should be grown at 37˚C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: HepG2 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

   Notes: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Replace media every 2-3 days until cells reach 90% confluency. At first passage and subsequent passages, use Thaw Medium 1.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 1 and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Thaw Medium 1.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:6 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 1 and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

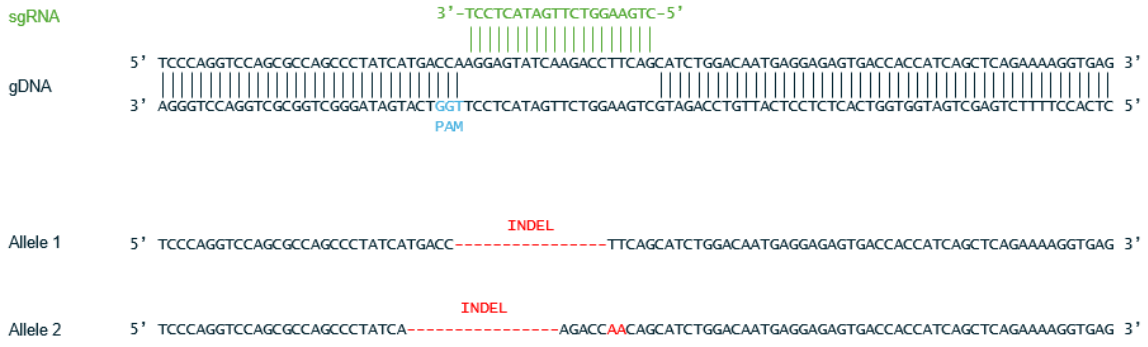

Figure 1. Genomic Sequencing of ASGR1 in the ASGR1 Knockout HepG2 Cell Line.
Genomic DNA from the ASGR1 Knockout HepG2 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (single guide RNA) in green, and the Indels (Insertions/Deletions) in the *ASGR1* alleles are indicated in red.

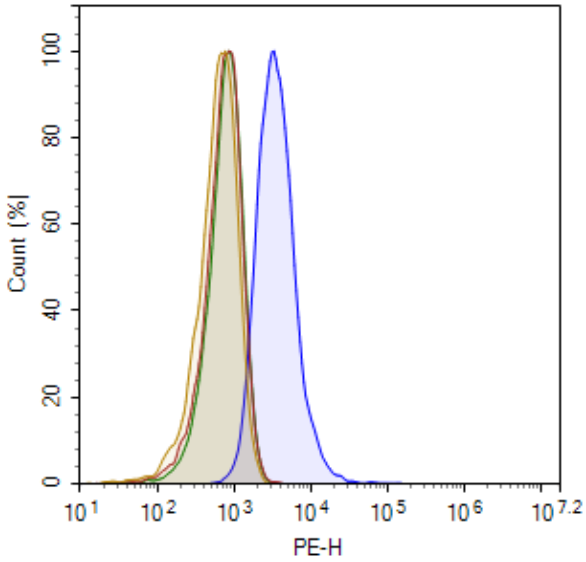

Figure 2. Expression of ASGR1 in the ASGR1 Knockout HepG2 Cell Line by flow cytometry.
Cells were stained with PE-labeled Rabbit anti-ASGR1 Polyclonal Antibody and analyzed by flow cytometry. Parental HepG2 cells are shown in blue, and ASGR1 Knockout HepG2 cells are shown in red. Unstained parental HepG2 cells are shown in yellow. As a control, parental HepG2 cells were stained with PE-labeled Donkey anti-Rabbit IgG Antibody and shown in green. The y-axis shows the % of cells, while the x-axis represents the fluorophore intensity.

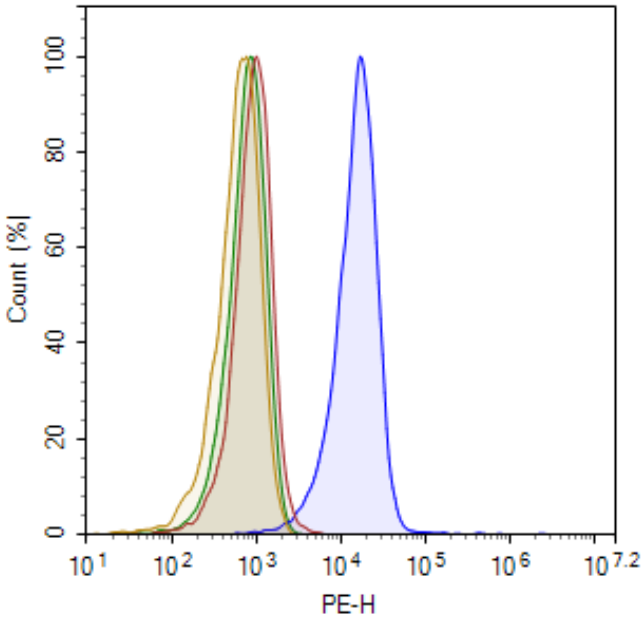

Figure 3. Cell surface expression of ASGR2 in the ASGR1 Knockout HepG2 Cell Line by flow cytometry.

The ASGPR complex present at the cell surface is composed of ASGR1 and ASGR2 subunits. Cells were stained with a PE-labeled anti-ASGR2 Recombinant Rabbit Monoclonal Antibody and analyzed by flow cytometry. Parental HepG2 cells are shown in blue, and ASGR1 Knockout HepG2 cells are shown in red. Unstained parental HepG2 cells are shown in yellow. As a control, parental HepG2 cells were stained with a PE-labeled Donkey anti-Rabbit IgG Antibody and are shown in green. The y-axis shows the % of cells, while the x-axis represents the fluorophore intensity. In the absence of ASGR1, ASGR2 is no longer detected on the cell surface.

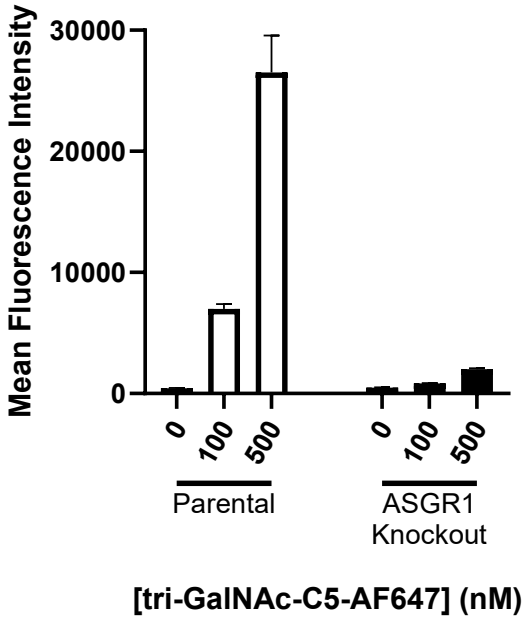

Figure 4. Uptake of a triantennary GalNAc-conjugated fluorescent ligand by ASGR1 Knockout HepG2 Cell Line.
The ASGPR complex ASGPR binds glycoproteins with a terminal galactose (Gal) or N-acetylgalactosamine (GalNAc) and targets them for endocytosis. HepG2 parental cells or ASGR1 Knockout HepG2 cells were seeded in a 24-well plate at 50,000 cells per well. The next day, cells were treated with either 0, 100, or 500 nM tri-GalNAc-C5-AF647 (R&D Systems #7901), a fluorescent ASGPR ligand. After 1 hour incubation at 37°C, cells were harvested and washed before measuring fluorescence by flow cytometry. Mean fluorescence intensity represents the level of tri-GalNAc-C5-AF647 endocytosed by the cells. Bars represent the mean of 3 replicates performed on the same day, and error bars show the standard deviation. This data is representative of multiple independent experiments.

Data shown is representative.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Notes
The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

References

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