Description

Recombinant stable HEK293 cell line constitutively expressing ZsGreen (bright green fluorescent protein derived from *Zoanthus sp*. reef coral) and firefly luciferase, which have been stably integrated into the AAVS1 safe harbor locus on chromosome 19 using CRISPR/Cas9. Expression of ZsGreen and the luciferase reporter are driven by a EF1A promoter (Figure 1). Cells were selected by limiting dilution to obtain a monoclonal population. The lack of random integration of ZsGreen/luciferase into the HEK293 genome guarantees this cell line behaves like parental HEK293 cells.

Figure 1: Schematic of the transgene integrated at the AAVS1 locus of chromosome 19.

Background

AAVS1 (also known as the PPP1R12C locus) on human chromosome 19 is a well-validated "safe harbor" site for hosting DNA transgenes. AAVS1 has an open chromatin structure and is transcription competent. Most importantly, disrupting the AAVS1 locus by inserting DNA transgenes using CRISPR/Cas9 has no known adverse effects on the cells. Specifically targeting the AAVS1 locus is a major advantage compared to the random integration obtained using other approaches such as lentivirus infection or cell transfection, which may cause insertional mutagenesis or disrupt important genes or cellular processes. The generation of a cell line containing both a fluorescent marker and luciferase allows for easy read outs in multiple assay types, ranging from *in vitro* to *in vivo* applications.

Application

- Use as a control in cell-based assays.
- Use as base cell line model for further genetic modifications of interest.

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

HEK293, Human Embryonic Kidney, epithelial-like cells, 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 6	BPS Bioscience #60183
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690

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 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: HEK293 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 6.

   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 6.

3. Transfer the resuspended cells to a T25 flask or T75 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 6 and continue growing the cells in a 5% CO_2 incubator at 37°C until ready to passage.

5. Cells should be passaged before they are fully confluent.

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 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 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 6.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 weekly, 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 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 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.

5. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

6. 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

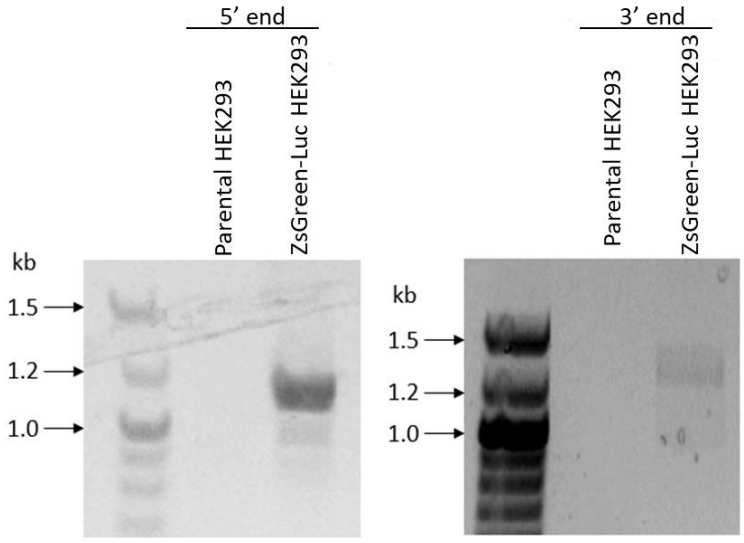

Figure 2: Polymerase chain reaction (PCR) amplification of the transgene integrated in the genome of HEK293 cells.
On the 5' end of the integration, the region spanning the chromosome 19 AAVS1 locus and the beginning of the promoter integration was amplified by PCR, with a predicted size of 1.1 kb. On the 3' end of the integration, the region spanning the SV40 Poly A signal region integration and the chromosome 19 AAVS1 locus was amplified by PCR, with a predicted size of 1.2 kb.

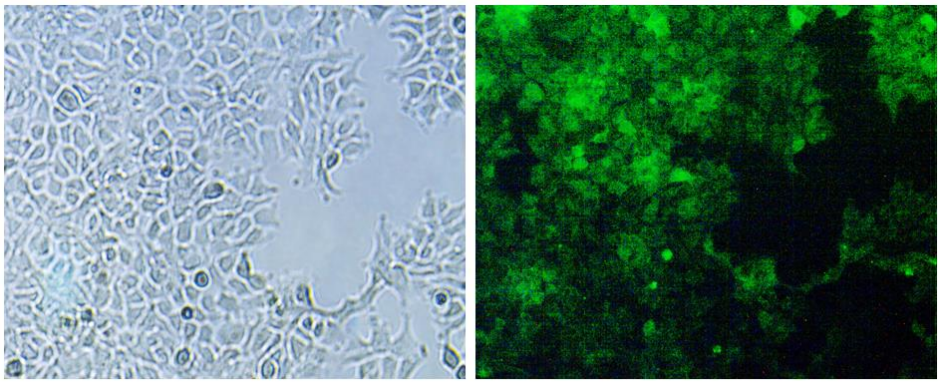

Figure 3: Expression of ZsGreen in the ZsGreen/Luciferase AAVS1 Safe Harbor HEK293 Cell Line. Bright field and fluorescence images of the ZsGreen/Luciferase AAVS1 Safe Harbor HEK293 cells were captured with a 10x magnification in a fluorescence microscope.

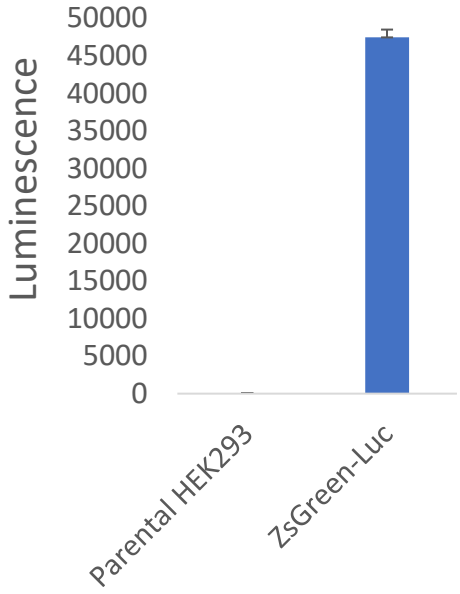

Figure 4. Luciferase activity in the ZsGreen/Luciferase AAVS1 Safe Harbor HEK293 Cell Line. ZsGreen/Luciferase Safe Harbor HEK293 cells or parental HEK293 cells were seeded in a 96-well plate at a density of 2 x 10^4 cells/well, and luciferase activity was measured with ONE-Step™ Luciferase Assay System.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

License Disclosure

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
RFP/GFP Safe-Harbor HEK293 Cell Line	78581	2 vials
Cas9/GFP Safe-Harbor Hek293 Cell Line	78582	2 vials
AAV-DJ ZsGreen	78442	50 µl x 2