Description

PARG Knockout HeLa Cell Line is a HeLa cell line in which human PARG (Poly ADP-ribose glycohydrolase) long isoforms ($PARG_{111}$, $PARG_{102}$ and $PARG_{99}$) have been genetically removed using CRISPR/Cas9 genome editing with a lentivirus encoding CRISPR/Cas9 gene and sgRNA (single guide RNA) targeting human PARG.

Background

Poly (ADP-ribose) glycohydrolase (PARG) is a catabolic enzyme involved in the degradation of PARylated chains, releasing ADP-ribose and oligo (ADP-ribose) chains. PAR (poly-ADP ribosylation) homeostasis is regulated by the family of PAR polymerases (PARPs) and PARG in response to cellular stress conditions such as DNA damage response (DDR). PARG activity is linked to cellular responses in inflammation, ischemia, stroke, and cancer. PARG is overexpressed in breast cancer and associated with tumor growth and survival. Decrease in PARG activity can potentiate the effect of current cancer therapies, such as chemotherapy and radiation, making PARG inhibition with selective inhibitors a promising approach in cancer and immunotherapy.

Application

- Use as a negative control when testing PARG inhibitors in HeLa cells.
- Study the phenotype of PARG knockout.
- Introduce further CRISPR/Cas9-based genetic manipulations in order to understand the interplay between PARG and other partners and pathways.

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)

Host Cell

HeLa human epithelial cell line. Adherent cells.

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 this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

Materials Required for Cell Line Culture

Name	Ordering Information
Thaw Medium 6	BPS Bioscience #60183

Storage Conditions

Cells will arrive 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 Line Culture

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

Cell Culture Protocol

Note: HeLa 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.

   Note: 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 24-48 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 6 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Continue to change medium every 2-3 days until cells are ready to passage.

6. Cells should be passaged before they reach 90% confluency. At first passage and subsequent passages, use Thaw Medium 6.

Cell Passage

1. Aspirate the medium, wash the cells twice with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}.

2. Treat cells with 2-3 ml of 0.25% Trypsin/EDTA and incubate for 2-3 minutes at 37°C.

3. Confirm cell detachment under a microscope.

4. Add 10 ml of pre-warmed Thaw Medium 6 and gently pipette up and down to dissociate cell clumps.

5. Transfer cells to a 15 ml conical tube and centrifuge at 200 *x g* for 5 minutes.

6. Remove the medium and resuspend cells in 10 ml pre-warmed Thaw Medium 6.

7. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10.

8. Incubate cells in a humidified 37°C incubator with 5% CO_2.

Cell Freezing

1. Aspirate the medium, wash the cells twice 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 6 and count the cells.

3. Spin down the cells at 200 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at 1~2 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 down at least 10 vials of cells at an early passage for future use.

Validation Data

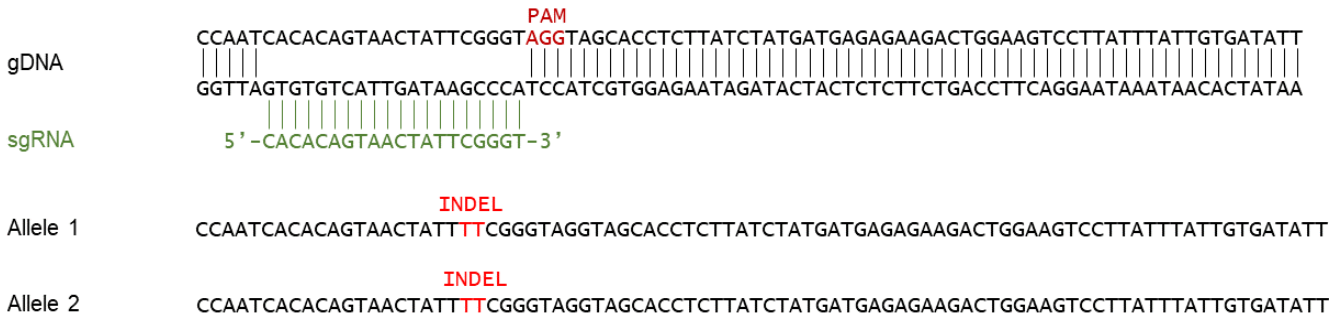

Figure 1. Genomic sequencing of PARG in the PARG Knockout HeLa Cell Line.
Genomic DNA from the PARG Knockout HeLa cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in maroon, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two PARG alleles are highlighted in red. The PARG genomic DNA is labeled as gDNA.

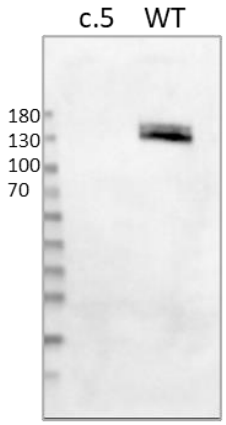

Figure 2. PARG Expression in PARG Knockout HeLa Cell Line by Western Blot.
Cell extracts of wild type (WT) HeLa cells and PARG Knockout HeLa cells (c.5) were analyzed by SDS-PAGE followed by Western Blot. PARG was detected with anti-PARG antibody (Proteintech #27808-1-AP).

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

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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.

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
Marques M., *et al.,* 2019 *Oncogene* 38 (12): 2177-2191.
James D. I., et al., 2016 *ACS Chem Biol* 11 (11): 3179-3190.
Drown B. S., *et al.*, 2018 *Cell Chem Bio 25* (12): 1562-1570.

Related Products

Products	*Catalog #*	*Size*
PARG, His-Tag Recombinant	101726	10 µg
PARG Fluorogenic Assay Kit	78858	96 reactions/384 reactions
LysA™ Universal PARylation Assay Kit	82123	96 reactions
LysA™ Protease Inhibitor Cocktail Kit	82199	1 kit
ADP-Ribosylation Cycle Inhibitor Mix	82130	5 x 20 µl

Version 041624