Description

PARP1 Knockout MDA-MB-231 Cell Line is an MDA-MB-231 cell line in which PARP1 (Poly-(ADP-ribose) Polymerase 1) has been genetically removed with CRISPR/Cas9 genome editing.

This cell line has been validated by genomic sequencing and Western Blot analysis.

Background

PARP1, also known as poly-(ADP-ribose) polymerase 1 or NAD+ ADP-ribosyltransferase 1, is part of the PARP family and the most abundant member. ADP ribosylation, which is the addition of an ADP-ribose to a protein, is a reversible post-translational modification of proteins mostly involved in the DNA Damage Response (DDR) pathway. Poly-ADP-ribosylation (termed PARylation) is the addition of linear or branched chains of ADP-ribose. PARP1 participates in DNA repair by non-homologous end joining (NHEJ), homologous recombination (HR), microhomology-mediated end-joining (MMEJ) and nucleotide excision repair. Dysfunction of DDR pathways can lead to oncogenesis. Overexpression of PARP1 has been found in breast and colon cancer, neuroblastoma, and others. This overexpression can lead to increasing MMEJ, an error-prone DNA repair mechanism, and genome instability leading to cancer. In addition to being involved in DDR, PARP1 is also linked to inflammation and type I diabetes. PARP1 inhibitors have been used in cancer treatment with success. In addition to reducing MMEJ, the use of PARP1 inhibitors can lead to synthetic lethality when homologous recombination repair (HRR) mechanisms are already defective, as in the case of BRCA1 (breast cancer susceptibility protein type 1) and BRCA2 deficient cells. Further understanding of the molecular pathways involving PARP1, and this contribution to disease, will continue to pave the way for new therapies for PARP1-linked diseases.

MDA-MB-231 is an epithelial cell line derived from a mammary gland adenocarcinoma, with mutant p53. It is ER (estrogen receptor), HER2, and E-cadherin negative, and it is used as a model of late-stage breast cancer.

Application

- Control cell line for testing inhibitors of PARP1 in MDA-MB-231 cells.
- Cellular model for studies on the role of PARP1.

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

MDA-MB-231, Human epithelial cells, breast adenocarcinoma, 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.

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Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 6	BPS Bioscience #60183

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℃ 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 12 (BPS Bioscience #78074)
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 1X MEM Non-essential Amino Acids.

Cell Culture Protocol

Note: MDA-MB-231 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 12.

   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 12.

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 12 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 12.

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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 12 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 12.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10 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 12 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 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

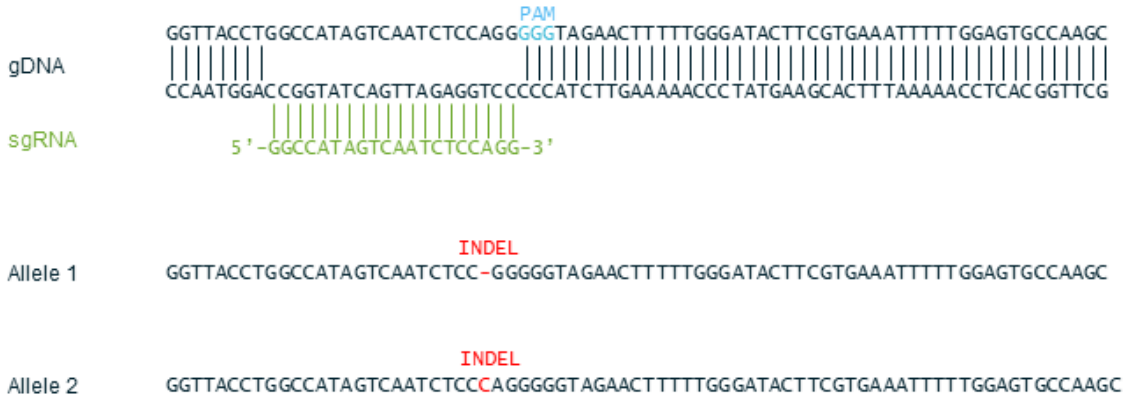

Figure 1: Genomic sequencing of PARP1 in the PARP1 Knockout MDA-MB-231 Cell Line.
Genomic DNA from the PARP1 Knockout MDA-MB-231 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two PARP1 alleles are highlighted in red. The PARP1 genomic DNA is labeled as gDNA.

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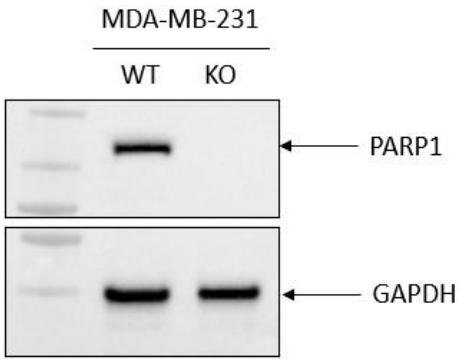

Figure 2: Expression of PARP1 in PARP1 Knockout MDA-MB-231 Cell Line by Western Blot. Parental MDA-MB-231 cells (WT) and PARP1 Knockout (KO) MDA-MB-231 cells lysates were run on a 4-20% SDS-PAGE gel and analyzed by Western Blot with anti-PARP1 Polyclonal Antibody (Thermo Fisher #PA5-34803). GAPDH was used as loading control.

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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.

Related Products

Products	*Catalog #*	*Size*
PARP1 Knockout HeLa Cell Line	82169	2 vials
LysA™ Universal PARylation Assay Kit	82123	96 reactions
ADP-Ribosylation Cycle Inhibitor Mix	82130	5 x 20 µl
LysA™ Protease Inhibitor Cocktail Kit	82199	1 kit
PARPtrap™ Assay Kit for PARP1	80584	96 reactions/384 reactions
PARP1 Chemiluminescent Assay Kit	80569	384 reactions

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