6405 Mira Mesa Blvd 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data Sheet

Cas9 Expressing MDA-MB-231 cell pool

Catalog #: 78069

Description

Cas9 (*Streptococcus pyogenes* CRISPR associated protein 9) is an endonuclease enzyme that, when recruited to a specific DNA sequence by the sgRNA (single guide RNA), introduces a double stranded break into the DNA. This double stranded break is repaired in mammalian cells either through Non-Homologous End Joining or Homologous Recombination. Non-Homologous End Joining often results in the deletion or insertion of several base pairs at the cut site, which, when resulting in a frameshift, causes the functional inactivation of the targeted gene. Cas9 expressing MDA-MB-231 cell pool can be transduced or electroporated with sgRNA targeting a gene of interest to quickly generate knock-out cell pools or cell lines.

Application

1. Quickly generating knock-out cell pools or cell lines in MDA-MB-231 cells.
2. Implementing sgRNA screens in Cas9 expressing MDA-MB-231 cells.

Format

Each vial contains ~2 x 10^6 cells in 1 ml of FBS with 10% DMSO.

Storage

Immediately upon receipt, store in liquid nitrogen.

Host Cell

MDA-MB-231 is a human breast cancer cell line from a patient with metastatic mammary adenocarcinoma. Adherent epithelial cells.

Culture Medium

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

Growth Medium 12A (BPS Bioscience #78075): DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 1X MEM Non-essential Amino Acids plus 0.25 µg/mL Puromycin to ensure recombinant expression.

6405 Mira Mesa Blvd 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Recommended Culture conditions

Frozen Cells: Prepare T-75 culture flask with 20 ml of pre-warmed Thaw Medium 12 (**no Puromycin**). Quickly thaw cells in a 37°C water bath with constant and slow agitation. After cleaning the outside of the vial with 70% ethanol, immediately transfer the entire content to Thaw Medium 12 (**no Puromycin**). Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO2. 24-48 hours after incubation, change to fresh Growth Medium 12A (**contains Puromycin**), without disturbing the attached cells. Continue to change medium every 2-3 days until cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture.

Subculture: When cells reached 90% confluency, remove the medium and wash twice with PBS (without Magnesium or Calcium). Treat cells with 2-3 ml of 0.25% Trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 mL pre-warmed medium and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and resuspend cells in 10 ml pre-warmed growth medium. Dispense 2 mL of the cell suspension into a new T75 flask containing pre-warmed 18 ml complete medium (a subcultivation ratio of 1:2 to 1:10 is recommended). Incubate cells in a humidified 37°C incubator with 5% CO_2.

Cryopreservation: When cells reach 90% confluency, spin cells, and remove medium from the pellet. Resuspend the cells in freezing medium (10% DMSO in FBS). Freeze cells using a reduced rate freezing box (-0.5°C to -1°C per minute) down to -80°C, then move cells to liquid nitrogen for long term storage. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

6405 Mira Mesa Blvd 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Validation
Expression of Cas9 was confirmed by flow cytometry.

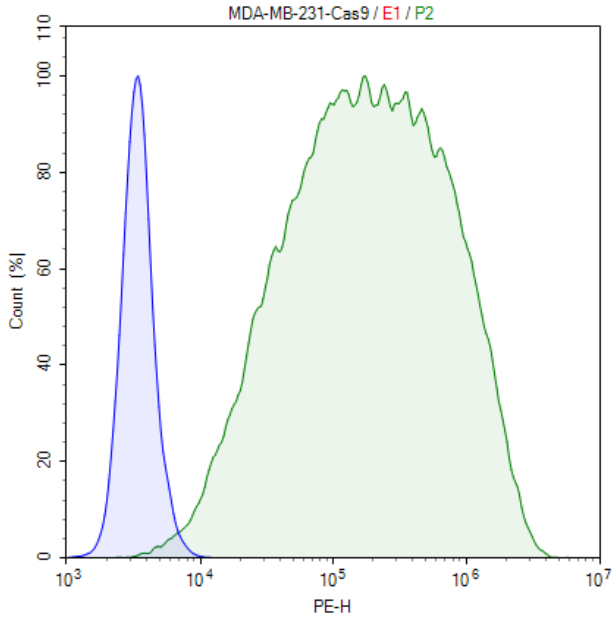

Figure 1. Expression of Cas9 in MDA-MB-231 cell pools.
Flow cytometry analysis of intracellular expression of Cas9 in MDA-MB-231 cell pools. Cell pools were stained with PE anti-FLAG antibody (BioLegend, #637309) and analyzed by FACS. Parental MDA-MB-231 cells are shown in blue, and the Cas9-expressing MDA-MB-231 cell pools are shown in green.

Mycoplasma Testing
This cell pool has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

6405 Mira Mesa Blvd 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Vector and Sequence

Streptococcus pyogenes Cas9, including a C-terminal FLAG tag, was transduced via lentivirus (BPS Bioscience, #78066).

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYH
EKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED
AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDE
HHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVK
LNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARG
NSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDR
FNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLK
RRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQ
SFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY
KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFF
YSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTG
GFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITI
MERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYV
NFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GDKRPAATKKAGQAKKKKDYKDDDDK

Related Products

Product	Cat. #	Size
Cas9 Expressing Jurkat cell pools	78070	2 vials
Cas9 Expressing Raji cell pools	78071	2 vials
Cas9 Expressing A549 cell pools	78072	2 vials
Cas9 Expressing HCT116 cell pools	78073	2 vials
Cas9 Lentivirus (puromycin selection)	78066	500 µl x 2
Cas9, His-tag (S. pyogenes)	100206-1	50 µg

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.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.