6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Cas9 Expressing A549 cell pool

Catalog #: 78072

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 A549 cells 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 A549 cells.
2. Implementing sgRNA screens in Cas9 expressing A549 cells.

Host Cell

Human lung alveolar vessel carcinoma cell line. Adherent epithelial 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.

Culture Medium

Thaw Medium 6 (BPS Bioscience, #60183): DMEM medium (Hyclone, #SH30243.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 6C (BPS Bioscience, #78077): Thaw Medium 6 (BPS Bioscience, #60183) plus 0.25 µg/mL Puromycin (Invivogen, #ant-pr-1) to ensure recombinant expression.

Culture Conditions

Thawing Cells: Prepare a 15 mL conical tube with 10 ml of pre-warmed Thaw Medium 6 (**no puromycin**). Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire contents to the conical tube. Spin cells at 200 x g for 5 minutes, and remove all medium from the pellet. Resuspend in 15mL Thaw Medium 6 (**no puromycin**) and transfer to a T-75 flask. Gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. 24 hours after incubation, change culture to fresh Thaw Medium 6 (**no puromycin**); avoid disturbing the

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attached cells. Continue to monitor growth for 2-3 days and change the media to remove dead cell debris, if necessary. Begin adding Growth Medium 6C after multiple cell colonies (in clumps) start to appear (indicative of healthy cell division).

Subculture: When cells have reached 90% confluency, remove Growth Medium 6C and gently wash cells twice with PBS (without Magnesium or Calcium). Treat cells with 2 ml of 0.25% Tryspin/EDTA and incubate for 2-3 minutes at 37°C. Dispense 10 ml of pre-warmed Growth Medium 6C to trypsinized cells and gently pipette up and down to neutralize trypsin and break apart any cell clumps. Transfer cells to a conical tube and centrifuge at 200 x g for 5 minutes. Remove Growth Medium 6C and re-suspend cells in 10-14 ml of prewarmed Growth Medium 6C. Dispense 2 ml of cell suspension into a new T-75 flask containing prewarmed 15 ml of Growth Medium 6C. Incubate cells in a humidified 37°C incubator with 5% CO_2.

Recommended split ratio: 1:5 to 1:7 twice per week.

Cryopreservation: When cells reach 90% confluency, use trypsin to remove cells from plate as above, 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.

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Validation

Expression of Cas9 was confirmed by flow cytometry.

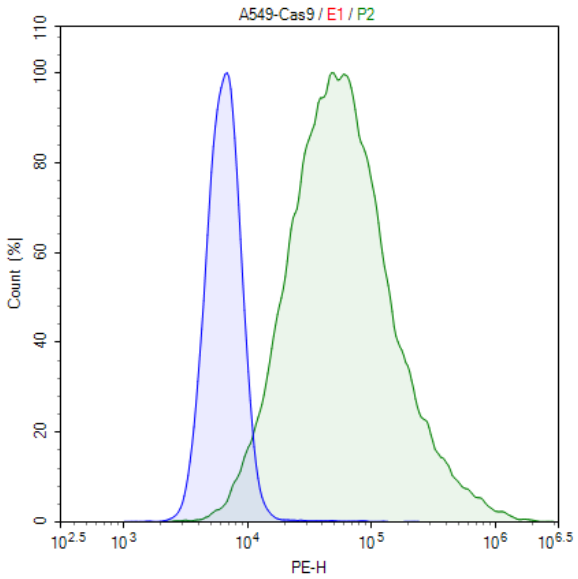

Figure 1. Expression of Cas9 in A549 cells.
Flow cytometry analysis of intracellular expression of Cas9 in A549 cells. Cells were stained with PE anti-FLAG antibody (BioLegend, #637309) and analyzed by FACS. Parental A549 cells are shown in blue, and the Cas9-expressing A549 cells are shown in green.

Mycoplasma Testing

This cell line 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.

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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 cells	78070	2 vials
Cas9 Expressing Raji cells	78071	2 vials
Cas9 Expressing MDA-MB-231 cells	78069	2 vials
Cas9 Expressing HCT116 cells	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.