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Data Sheet

Cas9 Expressing Raji cell pool

Catalog #: 78071

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 Raji cell pools 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 Raji cells.
2. Implementing sgRNA screens in Cas9 expressing Raji 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

Human B lymphoblastoid cell line, derived from a patient with Burkitt lymphoma.

Culture conditions

Thaw Medium 2 (BPS Bioscience, #60184): RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 2K (BPS Bioscience, #78078): RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.25 µg/ml of Puromycin to ensure recombinant expression.

Cells should be grown at 37˚C with 5% CO_2 using Growth Medium 2K to ensure recombinant expression is maintained.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37˚C water-bath, and then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 (**no Puromycin**). Then spin the cells down, remove the supernatant, and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2 (**no Puromycin**). Transfer the resuspended cells to a T25 flask

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and incubate at 37˚C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 3-4 ml of Thaw Medium 2 (**no Puromycin**). At first passage, switch to Growth Medium 2K (contains Puromycin). Cells should be split before they reach 2 x 10^6 cells/ml.

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.

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.

Validation
Expression of Cas9 was confirmed by flow cytometry.

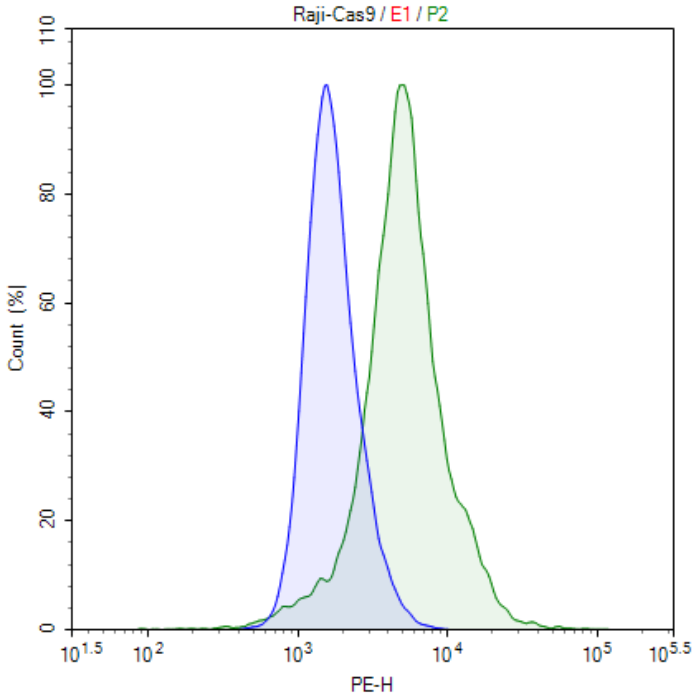

Figure 1. Expression of Cas9 in a Raji cell pool.
Flow cytometry analysis of intracellular expression of Cas9 in a Raji cell pool. The cell pool was stained with PE anti-FLAG antibody (BioLegend, #637309) and analyzed by FACS. Parental Raji cells are shown in blue, and the Cas9-expressing Raji cell pool is shown in green.

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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 cell pool	78070	2 vials
Cas9 Expressing MDA-MB-231 cell pool	78069	2 vials
Cas9 Expressing A549 cell pool	78072	2 vials
Cas9 Expressing HCT116 cell pool	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

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