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Data Sheet

Cas9 Expressing Jurkat cell pool

Catalog #: 78070

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 Jurkat 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 Jurkat cells.
2. Implementing sgRNA screens in Cas9 expressing Jurkat 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 conditions

Thaw Medium 2 (BPS Bioscience, #60184): RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 2K (BPS Bioscience, #78078): Thaw Medium 2 (BPS Bioscience, #60184) plus 0.25 µg/ml of Puromycin (Invivogen, #ant-pr-1) 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 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.

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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 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.

Validation
Expression of Cas9 was confirmed by flow cytometry.

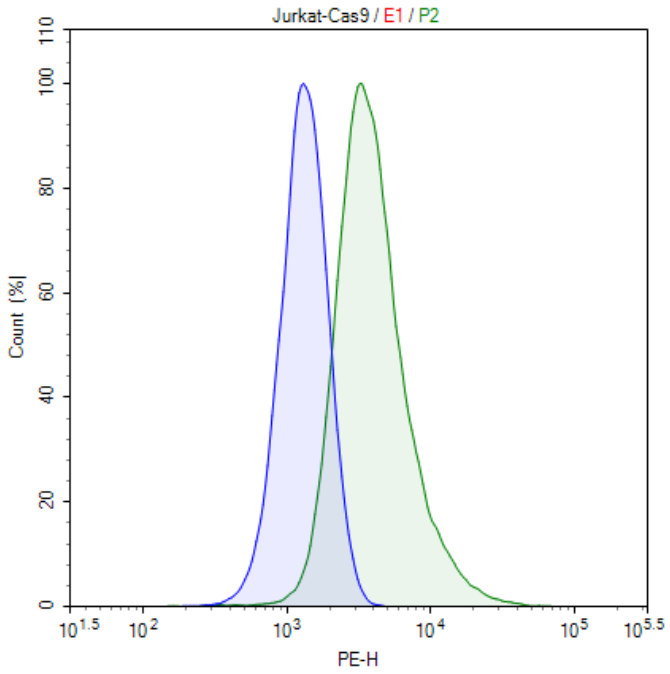

Figure 1. Expression of Cas9 in Jurkat cells.
Flow cytometry analysis of intracellular expression of Cas9 in Jurkat cells. Cells were stained with PE anti-FLAG antibody (BioLegend, #637309) and analyzed by FACS. Parental Jurkat cells are shown in blue, and the Cas9-expressing Jurkat cells are shown in 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 Raji cells	78071	2 vials
Cas9 Expressing MDA-MB-231 cells	78069	2 vials
Cas9 Expressing A549 cells	78072	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.