Description

This cell line is a clonal derivative from the Cas9-Expressing Jurkat Cell Pool (BPS Bioscience, #78070). It was generated by limiting dilution of the original pool and isolation of individual clones, which were screened based on Cas9 expression to obtain a high-expressing cell line. The expressed Cas9 protein includes a C-terminal FLAG tag.

Background

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 cell lines 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.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

Jurkat is a human leukemia cell line. Non-adherent T lymphocytes.

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.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

Materials Required for Cell Line Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2K	BPS Bioscience #78078

Storage Conditions

Cells will arrive upon 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.

License Disclosure

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Troubleshooting Guide

For all questions, please email support@bpsbioscience.com.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2K.

Media Required for Cell Line Culture

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.

Recommended Culture Conditions

Frozen cells:

1. 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 pre-warmed Thaw Medium 2 (**no Puromycin**).
2. Then spin the cells down, remove the supernatant, and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2 (**no Puromycin**).
3. Transfer the resuspended cells to a T25 flask and incubate at 37˚C in a 5% CO_2 incubator.
4. After 24 hours of culture, add an additional 3-4 ml of Thaw Medium 2 (**no Puromycin**).
5. At first passage, switch to Growth Medium 2K (contains Puromycin).
6. Cells should be split before they reach 2 x 10^6 cells/ml.

Cryopreservation:

1. When cells have reached 90% confluency, spin cells and remove medium from the pellet.
2. Resuspend the cells in freezing medium (10% DMSO in FBS).
3. 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.

Validation Data

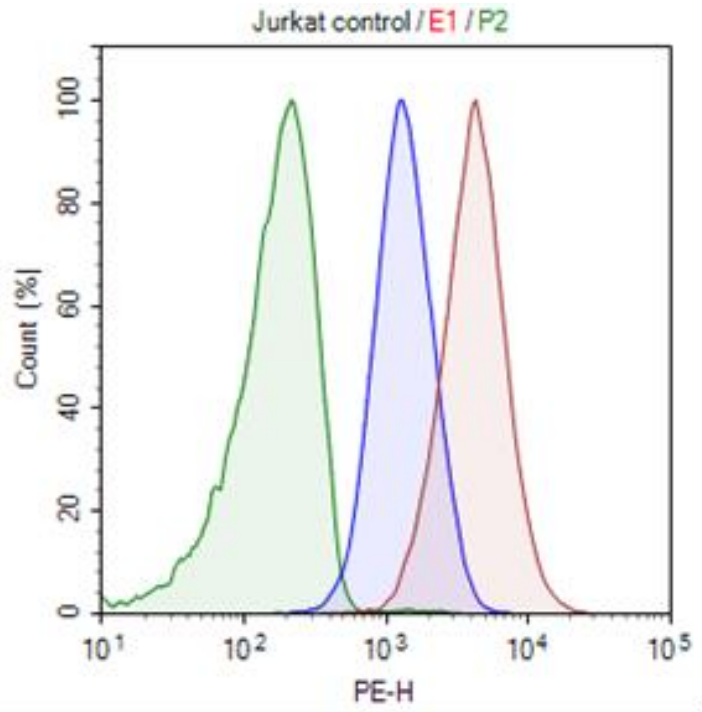

Figure 1. Flow cytometry analysis of intracellular expression of Cas9 in Jurkat cells.
Cells were stained with PE-labeled anti-FLAG antibody (BioLegend, #637309) and analyzed by flow cytometry. The parental Jurkat cells are shown in green, the Cas9-expressing Jurkat High expression cell line (BPS Bioscience, #78136-H) is shown in red, and the Cas9-expressing Jurkat Low expression cell line (BPS Bioscience, #78136-L) is shown in blue.

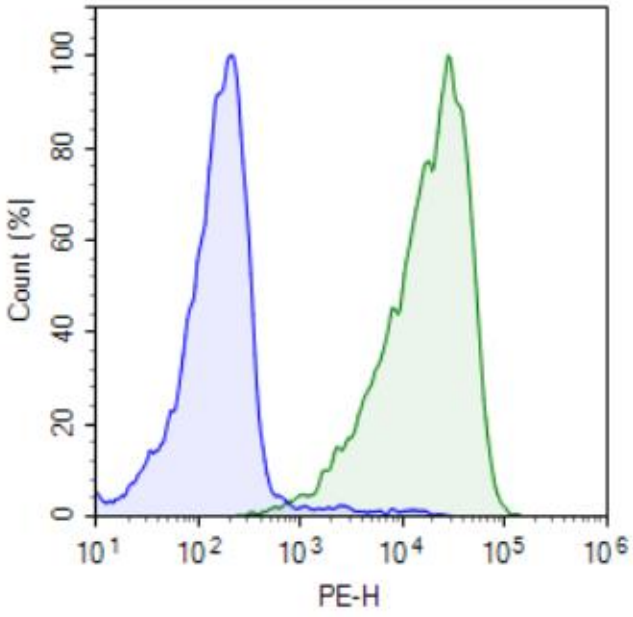

Figure 2. Flow cytometry analysis of TCR expression following knock-out in Cas9-expressing Jurkat cells. Cas9-expressing cells (BPS Bioscience, #78136-H) were electroporated with 0.1 nmol TCR sgRNA, and TCR expression was analyzed by flow cytometry 72 hours later. The parental Cas9-expressing Jurkat High expression cell line (BPS Bioscience, #78136-H) is shown in green, and the TCR knock-out cell pool is shown in blue.

Vector and Sequence

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

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSN
EMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP
DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKL
QLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKI
EKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKV
KYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL
EDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLT
FKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGI
KELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEE
VVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS
KLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFK
TEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF
DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG
NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHL
FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKDYKDDDDK

Related Products

Products	*Catalog #*	*Size*
Cas9-Expressing Jurkat cell pool	78070	2 vials
Cas9 Lentivirus (puromycin selection)	78066	500 µl x 2
Cas9, His-tag (S. pyogenes)	100206-1	50 µg
Thaw Medium 2	60184-1	100 ml
Growth Medium 2K	78078	500 ml

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.