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

Materials Provided

Components	Format
2 vials of frozen cell pools	2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

Daudi cells are a Burkitt's lymphoma B-cell line. Suspension cells.

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 Pool 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.

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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 1K.

Media Required for Cell Pool 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 3B (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.

Cell Pool Culture Protocol

Cell Thawing

1. Prepare a 15 ml conical tube with 10 ml of pre-warmed Thaw Medium 2 (no Puromycin).
2. Quickly thaw cells in a 37°C water bath with constant and slow agitation.
3. Clean the outside of the vial with 70% ethanol and immediately transfer the entire contents to the conical tube containing 10 ml of Thaw Medium 2 (**no Puromycin**).
4. Spin the cells down at 200 x g for 5 minutes, remove the supernatant, and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2 (**no Puromycin**).
5. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.
6. After 24 hours of culture, add an additional 3-4 ml of Thaw Medium 2 (**no Puromycin**).
7. At first passage, switch to Growth Medium 2K (contains Puromycin). Cells should be split before they reach 2 x 10^6 cells/ml.

Cryopreservation

1. When cells reach 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.
4. 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 Data

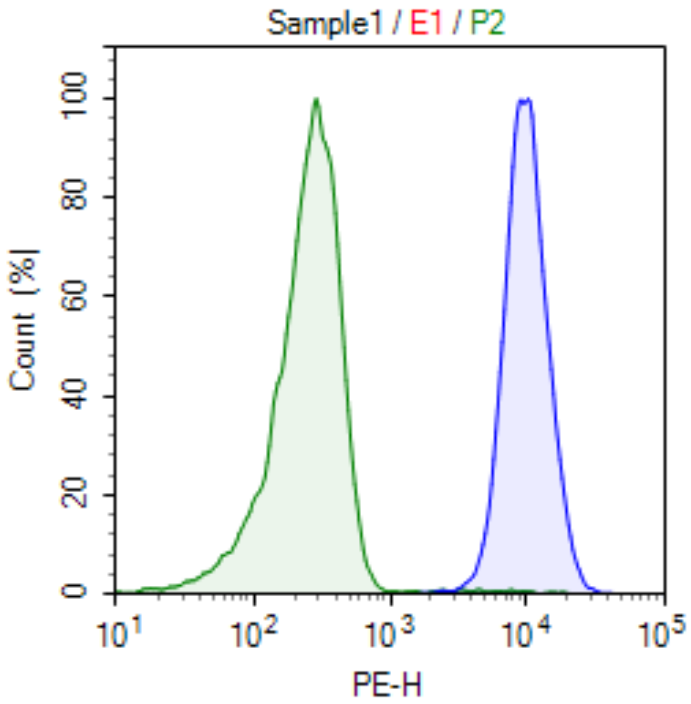

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Figure 1. Expression of Cas9 in Daudi cellsFlow cytometry analysis of intracellular expression of Cas9 in Daudi cell pool. Cells were stained with PE-labeled anti-FLAG antibody (BioLegend, #637309) and analyzed by FACS. Parental Daudi cells are shown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

Related Products

Products	*Catalog #*	*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 Expressing Neuro2a cells	78087	2 vials
Cas9 Expressing Jurkat cells	78070	2 vials
Cas9 Lentivirus (Puromycin selection)	78066	500 µl x 2
Cas9 Lentivirus (Hygromycin selection)	78067	500 µl x 2
Cas9, His-tag (*S. pyogenes*)	100206-1	50 µg

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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.

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