CRISPRa (SAM) Jurkat Cell Line

Description

This cell line has been engineered for use with the CRISPR Synergistic Activation Mediator (SAM) system to induce transcriptional activation and expression of any gene of interest. Cells stably express a mutated dCas9 (Streptococcus pyogenes CRISPR associated protein 9), lacking any endonuclease activity, fused to VP64, a transcriptional activator. Stable dCas9-VP64 expression is maintained with Blasticidin resistance. Cells also stably express P65 (Transcription Factor p65, or Nuclear Factor NF-κ-B p65) and HSF1 (Heat Shock Factor 1) fused with an MS2 tag, which is maintained with Hygromycin resistance. When these cells are transfected with an MS2-tagged sgRNA targeting the promoter region of the gene of interest, dCas9-VP64 and MS2-P65-HSF1 are recruited to the site in the genomic DNA and begin transcription, inducing expression of the desired gene.

Application

1. Quickly generating CRISPR-activated cell pools or cell lines in Jurkat cells
2. Implementing sgRNA CRISPRa screens in 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

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 2L	BPS Bioscience #78094

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.

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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 2L.

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 2L (BPS Bioscience #78094):

Thaw Medium 2 (BPS Bioscience, #60184) plus 200 µg/ml of Hygromycin B (Invivogen, #ant-hg) and 5 ug/ml Blasticidin (Invivogen, #ant-bl) to ensure recombinant expression.

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

Recommended Culture Conditions

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 Thaw Medium 2 (**no Hygromycin or Blasticidin**).
2. Then spin the cells down, remove the supernatant, and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2 (**no Hygromycin or Blasticidin**).
3. Transfer the resuspended cells to a T25 flask and incubate at 37˚C in a 5% CO2 incubator.
4. After 24 hours of culture, add an additional 3-4 ml of Thaw Medium 2 (**no Hygromycin or Blasticidin**).
5. At first passage, switch to Growth Medium 2L (contains Hygromycin and Blasticidin).
6. 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, 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 Data

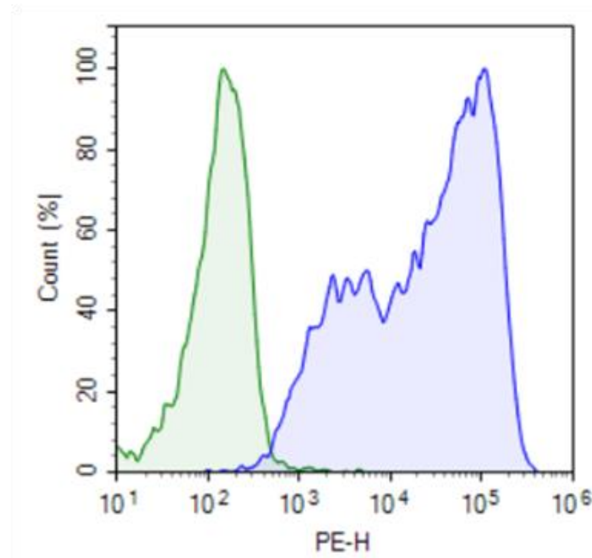

Figure 1. Induction of PD-1 in CRISPRa (SAM) Jurkat cells.
CRISPRa (SAM) Jurkat cells were electroporated with 1 ug sgRNA-MS2 plasmid targeting PD-1 (Programmed Cell Death protein 1, or CD279, BPS Bioscience #78091) to induce PD-1 expression. Cells were stained 72 hours post-electroporation (with no selection) with PE-labeled anti-PD-1 antibody (BioLegend, #637309) and analyzed by FACS. Parental CRISPRa (SAM) Jurkat cells are shown in green, and the transfected CRISPRa (SAM) Jurkat cells are shown in blue.

Vector and Sequence

dCas9 (nuclease deficient Streptococcus pyogenes Cas9, in blue) fused with a linker (black) and VP64 (red):

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPD
NSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQ
LSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD
QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIE
KILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVK
YVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILE
DIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTF
KEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIK
ELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEV
VKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKL
VSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTE
ITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFD
SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN
ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSAGGGGSGGGGSGGGGSGPKKKRKVAAAGSGR
ADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDML

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MS2 (in blue) fused with a linker (black), P65 (red), and HSF1 (green):

ASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQKRKYTIKVEVPKVATQTVGGVELPVAAWR
SYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYSAGGGGSGGGGSGGGGSGPKKKRKVAAAGSPSGQISNQA
LALAPSSAPVLAQTMVPSSAMVPLAQPPAPAPVLTPGPPQSLSAPVPKSTQAGEGTLSEALLHLQFDADEDLGALLGNSTDPGVF
TDLASVDNSEFQQLLNQGVSMSHSTAEPMLMEYPEAITRLVTGSQRPPDPAPTPLGTSGLPNGLSGDEDFSSIADMDFSALLSQI
SSSGQGGGGSGFSVDTSALLDLFSPSVTVPDMSLPDLDSSLASIQELLSPQEPPRPPEAENSSPDSGKQLVHYTAQPLFLLDPGSVD
TGSNDLPVLFELGEGSYFSEGDGFAEDPTISLLTGSEPPKAKDPTVS

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 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.

Reference

Konermann, S., et al. (2015). Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature. 517(7536): 583-588.