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Data Sheet

CRISPRa (SAM) Jurkat Cell Line

Catalog #: 78080

Descriptions

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.

Format

Each vial contains ~2 x 10^6 cells in 1 ml of FBS with 10% DMSO.

Storage

Store in liquid nitrogen immediately upon receipt.

Culture conditions

Thaw Medium 2 (BPS Bioscience, #60184): RPMI 1640 medium (ThermoFisher, #A1049101) supplemented with 10% FBS (ThermoFisher, #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.

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**). 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**). 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 Hygromycin or**

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Blasticidin). At first passage, switch to Growth Medium 2L (contains Hygromycin and Blasticidin). 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 at a low passage number 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 dCas9-VP64 and MS2-P65-HSF1 was functionally validated by transfecting the cells with sgRNA-MS2 targeting PD-1.

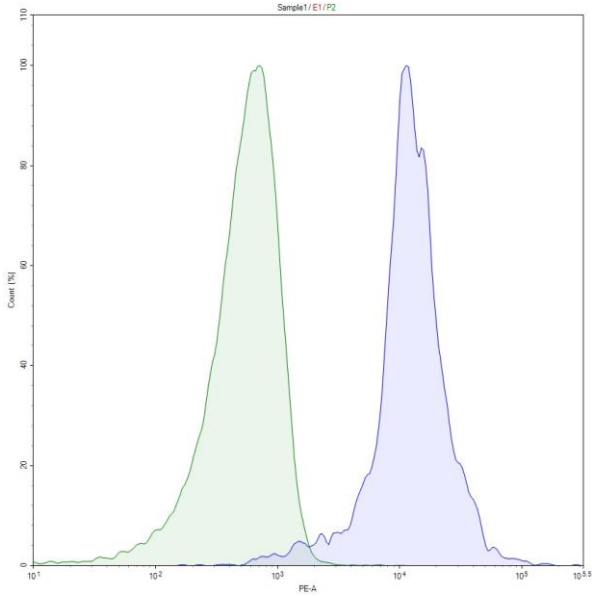

Figure 1. Induction of PD-1 in CRISPRa (SAM) Jurkat cells.
CRISPRa (SAM) Jurkat cells were electroporated with sgRNA-MS2 targeting PD-1 (Programmed Cell Death protein 1, or CD279, BPS Bioscience #78091) to induce PD-1 expression. Cells were stained with PE-labeled anti-PD-1 antibody (BioLegend, #637309) and analyzed by FACS. Parental CRISPRa (SAM) Jurkat cells are shown in green, and the transfected CRISPRa (SAM) Jurkat cells are shown in blue.

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Sequence

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

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRT
ARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEK
YPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE
ENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAK
LQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHH
QDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN
REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNS
RFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNEL
TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNA
SLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRR
YTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDS
LHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRI
EEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLK
DDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMER
SSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL
YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDS
AGGGGSGGGGSGGGGSGPKKKRKVAAAGSGRADALDDFDLDMLGSDALDDFDLDMLGSDA
LDDFDLDMLGSDALDDFDLDML

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

ASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQKRKYTIKVE
VPKVATQTVGGVELPVAAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGI
YSAGGGGSGGGGSGGGGSGPKKKRKVAAAGSPSGQISNQALALAPSSAPVLAQTMVPSSAM
VPLAQPPAPAPVLTPGPPQSLSAPVPKSTQAGEGTLSEALLHLQFDADEDLGALLGNSTDPGV
FTDLASVDNSEFQQLLNQGVSMSHSTAEPMLMEYPEAITRLVTGSQRPPDPAPTPLGTSGLPN
GLSGDEDFSSIADMDFSALLSQISSSGQGGGGSGFSVDTSALLDLFSPSVTVPDMSLPDLDSSL
ASIQELLSPQEPPRPPEAENSSPDSGKQLVHYTAQPLFLLDPGSVDTGSNDLPVLFELGEGSYF
SEGDGFAEDPTISLLTGSEPPKAKDPTVS

Reference

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

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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.

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