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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<sup>6</sup> 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 µg/ml Blasticidin (Invivogen, #ant-bl) to ensure recombinant expression.

Cells should be grown at 37°C with 5% CO<sub>2</sub> 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<sub>2</sub> incubator. After 24 hours of culture, add an additional 3-4 ml of Thaw Medium 2 (**no Hygromycin or Blasticidin**).

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**Blasticidin**). At first passage, switch to Growth Medium 2L (contains Hygromycin and Blasticidin). Cells should be split before they reach  $2 \times 10^6$  cells/ml.

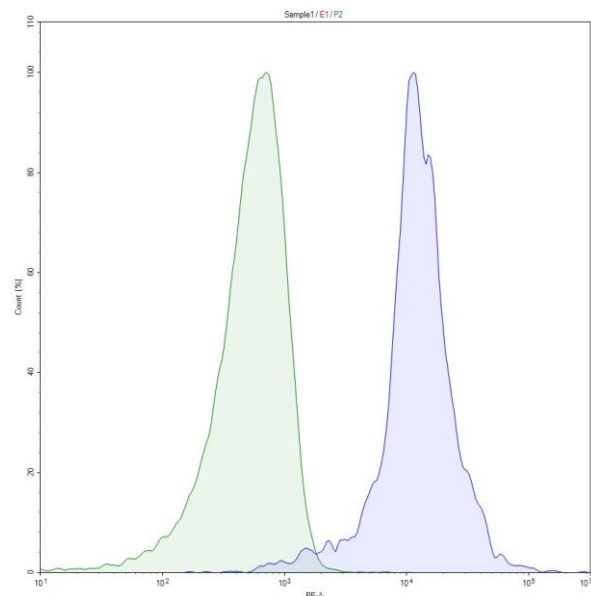
**Cryopreservation**: When cells reach 90% confluency, spin cells, and remove medium from the pellet. Resuspend the cells in freezing medium (10% DMSO in FBS). Freeze cells using a reduced rate freezing box ( $-0.5^{\circ}\text{C}$  to  $-1^{\circ}\text{C}$  per minute) down to  $-80^{\circ}\text{C}$ , 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):

DKKYSIGLAIGTNSVGVAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRT  
 ARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEK  
 YPTIYHLRKKLV DSTDKADLR LIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFE  
 ENPINASGVDAKAILSARLSKSRRLLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDAK  
 LQLSKD TYDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHH  
 QDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNL  
 REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTRIPYVVGPLARGNS  
 RFAWMTRKSEETITPWNFEEVVDK GASAQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNEL  
 TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNA  
 SLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRR  
 YTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDS  
 LHEHIANLAGSPAIKKGIQTVKVVDLKVVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRI  
 EEGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLK  
 DDSIDNKVLRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL  
 DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVR  
 EINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVVYDVRKMIKSEQEIGKATAKYFFYSN  
 IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNVKKTEVQTGGFS  
 KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEK GKSKKLKSVKELLGITIMER  
 SSFEKNPIDFLEAKGYKEVKKDLIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL  
 YLASHYEKLGKSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL SAYNKHRDKPI  
 REQAENIIHLFTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI TGLYETRIDLSQLGGDS  
 AGGGGSGGGGSGGGGSGPKKKR KVAAAGSGRADALDDFDL DMLGSDALDDFDL DMLGSDA  
 LDDFDL DMLGSDALDDFDL DML

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

ASNFTQFVLVDNNGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQKRKYTIKVE  
 VPKVATQTVGGVELPVAAWRSYLN MELTIPIFATNSDCELIVKAMQGLLDGNPIPSAIAANSI  
 YSAGGGGSGGGGSGGGGSGPKKKR KVAAAGSPSGQISNQALALAPSSAPVLAQTMVPSSAM  
 VPLAQPPAPAPVLTGPPQSL SAPVPKSTQAGEGTLSEALLHLQF DAEDELGALLGNSTDPGV  
 FTDLASVDNSEFQQLLNQGVSM SHSTAEPMLMEYPEAITRLVTGSQRPPDPAPTPLGTSGLPN  
 GLSGDEDFSSIADMDFSALLSQISSGQGGGGSGF SVDT SALLDLFSPSVTPDMSLPDL DSSL  
 ASIQELLSPQEPPRPEAENSSPDSGKQLVHYTAQPLFLLDPGSVDTGSNDLPVLFELGEGSYF  
 SEGDFGAEDPTISLLTGSEPPKAKDPTVS

**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

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
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.*

## License Disclosure

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