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-κB 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 HepG2 cells
2. Implementing sgRNA CRISPRa screens in HepG2 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 Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1S	BPS Bioscience #78221

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.

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

Media Required for Cell Culture

Thaw Medium 1(BPS Bioscience # 60187):

MEM medium (Thermo Fisher, #11095098) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163).

Growth Medium 1S (BPS Bioscience # 78221):

Thaw Medium 1 (BPS Bioscience # 60187) plus 200 µg/ml of Hygromycin B (Thermo Fisher, #10687010) and 5 µg/ml Blasticidin (Invivogen, #ant-bl-1) to ensure recombinant expression.

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 1 (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 1 (no Hygromycin or Blasticidin).
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 1S (no Hygromycin or Blasticidin).
5. At first passage, switch to Growth Medium 1S (contains Hygromycin and Blasticidin)

Subculture:

1. When cells have reached 90% confluency, remove the growth medium and wash twice with PBS (without Magnesium or Calcium).
2. Add 2-3 ml of 0.25% Trypsin/EDTA to the cells and incubate for 2-3 minutes at 37°C.
3. After confirming cell detachment by light microscopy, add 10 mL pre-warmed Growth Medium 1S and gently pipette up and down to dissociate cell clumps.
4. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes.
5. Remove the medium and resuspend cells in 10 ml pre-warmed Growth Medium 1S.
6. Dispense 2 mL of the cell suspension into a new T75 flask containing pre-warmed 18 ml Growth Medium 1S (a subcultivation ratio of 1:2 to 1:10 is recommended).
7. Incubate cells in a humidified 37°C incubator with 5% CO_2.

Cryopreservation:

1. When cells have reached 90% confluency, use trypsin to remove cells from plate as described above, 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 transfer 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

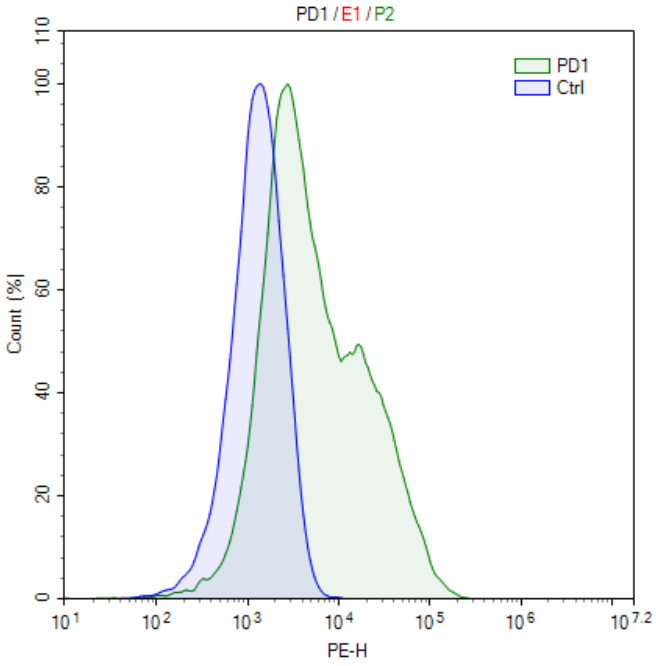

Figure 1. Induction of PD-1 in CRISPRa (SAM) HepG2 cells.
CRISPRa (SAM) HepG2 cells were transduced with sgRNA-MS2 lentivirus targeting PD-1 (BPS Bioscience, #78190) to induce PD-1 expression. Cells were stained 72 hours post-electroporation (with no selection) with PE-labeled anti-PD-1 antibody (BioLegend, #637309) and analyzed by flow cytometry. Parental CRISPRa (SAM) HepG2 cells are shown in blue, and the transfected CRISPRa (SAM) HepG2 cells are shown in green.

Vector and Sequence

dCas9 (nuclease deficient Streptococcus pyogenes Cas9, in blue) fused with a linker (black) and VP64 (red):
DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHELAYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP
DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKL
QLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKI
EKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKV
KYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL
EDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLT
FKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGI
KELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEE
VVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS
KLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFK
TEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF
DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG
NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHL
FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSAGGGGSGGGGSGGGGSGPKKKRKVAAAGS
GRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDML

MS2 (in blue) fused with a linker (black), P65 (red), and HSF1 (green):
ASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQKRKYTIKVEVPKVATQTVGGVELPVAAWR
SYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYSAGGGGSGGGGSGGGGSGPKKKRKVAAAGSPSGQISNQA
LALAPSSAPVLAQTMVPSSAMVPLAQPPAPAPVLTPGPPQSLSAPVPKSTQAGEGTLSEALLHLQFDADEDLGALLGNSTDPGVF
TDLASVDNSEFQQLLNQGVSMSHSTAEPMLMEYPEAITRLVTGSQRPPDPAPTPLGTSGLPNGLSGDEDFSSIADMDFSALLSQI
SSSGQGGGGSGFSVDTSALLDLFSPSVTVPDMSLPDLDSSLASIQELLSPQEPPRPPEAENSSPDSGKQLVHYTAQPLFLLDPGSVD
TGSNDLPVLFELGEGSYFSEGDGFAEDPTISLLTGSEPPKAKDPTVS

Products	*Catalog #*	*Size*
CRISPRa (SAM) HeLa Cell Line	78193	2 vials
CRISPRa (SAM) HEK293 Cell Line	78192	2 vials
CRISPRa (SAM) Jurkat Cell Line	78080	2 vials
PD-1 sgRNA-MS2 Lentivirus (Integrating)	78190	2 vials
PD-1 sgRNA-MS2 for CRISPRa (Plasmid)	78091	5 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.* Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex*. Nature.* 2015*.* **517(7536):** 583-588*.*