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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 a construct consisting of P65 (Transcription Factor p65, or Nuclear Factor NF-κ-B p65) and HSF1 (Heat Shock Factor 1) fused with an MS2 tag (from the MS2 coat protein), which is maintained with Hygromycin resistance. When these cells are transfected with an MS2 aptamer fused to a single guide RNA (sgRNA) targeting the promoter region of the gene of interest, dCas9-VP64 and MS2-P65-HSF1 are recruited to the promoter in the genomic DNA by the MS2 aptamer and begin transcription, inducing high level expression of the desired protein.

Cells were functionally validated by transfecting the cells with an sgRNA-MS2 targeting PD-1, resulting in high expression of PD-1.

Application(s)

- Quickly generate CRISPR-activated cell pools or cell lines in MCF7 cells.
- Implement sgRNA CRISPRa screens in MCF7 cells.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2×10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

MCF7 cells, human breast mammary gland cell line, epithelial cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 3N	BPS Bioscience #78534

Storage Conditions

Cells are shipped in 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.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.



Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO₂. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin

Growth Medium 3N (BPS Bioscience #78534):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1200 µg/ml Hygromycin B and 5 µg/ml Blasticidin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1 (**no Blasticidin or Hygromycin**).
Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.
2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1 (**no Blasticidin or Hygromycin**).
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 (**no Blasticidin or Hygromycin**) and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium (**contains Blasticidin and Hygromycin**).

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3N and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium (**contains Blasticidin and Hygromycin**). Seed into new culture vessels at the desired sub-cultivation ratio of 1:2 to 1:10.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

- Once the cells have detached, add Growth Medium 3N and count the cells.
- Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at $\sim 2 \times 10^6$ cells/ml.
- Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
- Transfer the vials to liquid nitrogen the next day for storage.



Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

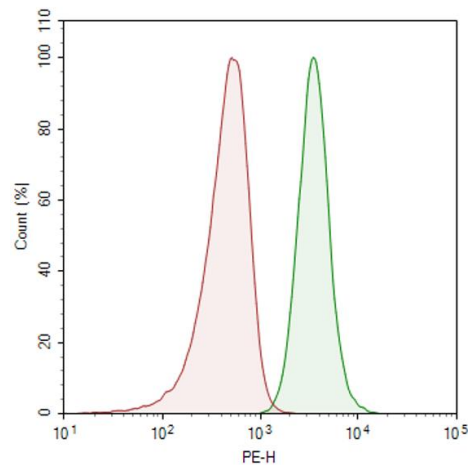


Figure 1. Induction of PD-1 in CRISPRa (SAM) MCF7 cells.

CRISPRa (SAM) MCF7 cells were transduced with PD-1 (Human) sgRNA-MS2 Lentivirus (BPS Bioscience #78190) to induce PD-1 expression. Cells were stained with PE-labeled anti-PD-1 antibody (BioLegend #637309) and analyzed by flow cytometry. Parental CRISPRa (SAM) MCF7 cells are shown in red, and the transduced CRISPRa (SAM) MCF7 cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

Sequence

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

DKKYSIGLAIGTNSVGVAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRNKRICYLQEIFSNE
 MAKVDDSFHRLVESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHMIKFRGHFLIEGDLNPD
 NSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQ
 LSKDXYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFD
 QSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNREDLLRQRTFDNGSIPHQIHLGELHAILRRQEDFYPLKDNREKIE
 KILTRIPYVYVGPLARGNSRFAMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVK
 YVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILE
 DIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTF
 KEDIQKAQVSGQGDSLHEHIANLAGSPAIKKILQTVKVVDELVKVMGRHKPENIVEMARENQTTQKGQKNSRERMKRIIEGK
 ELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLRSDKARGKSDNVPSEEV

VKKMKNYWRQLLNAKLITQRKFDNLTKAERGGSELKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS
 VSDFRKDFQFYKVINNYHHAHDAYLNAVVGALIKKYPKLESEFVYGDYKVVYDVRKMIKSEQEIGKATKAYFFYSNIMNFFKTE
 ITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIIVKKEVQQTGGFSKESILPKRNSDKLIARKKDWDPKYYGGFD
 SPTVAYSVLVVAKVEKGSKKLKSVELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIILPKYSLFELENGRKRMLASAGELQKGN
 ELALPSKYVNFYLYLASHYEKLGKSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHRDKPIREQAENIIHLFT
 LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSTIGLYETRIDLSQLGGDSAGGGGSGGGGSGGGGSGPKKKRKRVAAGSGR
 ADALDDFDLMLGSDALDDFDLMLGSDALDDFDLMLGSDALDDFDLML

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

ASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQKRKYTIKVEVPKVATQTVGGVELPVAAWR
 SYLNMELTIPIFATNSDCELIVKAMQGLLDGNPIPSAIAANSIY SAGGGGSGGGGSGGGGSGPKKKRKRVAAGSPSGQISNQA
 LALAPSSAPVLAQTMVPSSAMVPLAQPPAPVLTGPPQSLAPVPKSTQAGEGLSEALLHLQFADADLGLLNSTDPGVF
 TDLASVDNSEFQQLLNQGVSMHSTAEPMLMEYEAITRLVTGSQRPPDPAPTPLGTSGLPNGLSGDEDFSSIADMDFSALLSQI
 SSSGQGGGSGFSVDTALLDFSPSVTPDMSLPDLSSLASIQELLSPQEPPEAENSSPDSGKQLVHYTAQPLFLDPPGSD
 TGSNDLPVLFELGEGSYFSEGDGFAEDPTISLLTGSEPPKAKDPTVS

References

Konermann, S., *et al.* (2015) *Nature*. **517(7536)**: 583-588

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
CRISPRa (SAM) HeLa Cell Line	78193	2 vials
CRISPRa (SAM) HEK293 Cell Line	78192	2 vials
CRISPRa (SAM) Jurkat Cell Line	78080	2 vials
CRISPRa (SAM) HepG2 Cell Line	78194	2 vials