

Description

The binding of Programmed Cell Death Protein 1 (PD-1), a receptor expressed on activated T-cells, to its ligands, PD-L1 and PD-L2, negatively regulates immune responses. PD-1 ligands are found on most cancers, and the PD-1:PD-L1/2 interaction inhibits T-cell activity and enables cancer cells to escape immune surveillance. The PD-1:PD-L1/2 pathway is also involved in regulating autoimmune responses, making these proteins promising therapeutic targets for a number of cancers, as well as multiple sclerosis, arthritis, lupus, and type I diabetes.

This sgRNA (single guide RNA) pool is designed for use with the CRISPR Synergistic Activation Mediator (SAM) system to induce transcriptional activation and expression of any gene of interest. CRISPRa (SAM) cells stably express a mutated dCas9 (Streptococcus pyogenes CRISPR associated protein 9), lacking any endonuclease activity, fused with VP64, a transcriptional activator, as well as transcription factors P65 (Transcription Factor p65, or Nuclear Factor NF- κ -B p65) and HSF1 (Heat Shock Factor 1) fused with an MS2 tag.

When these cells are transfected with an MS2-tagged sgRNA targeting the promoter region of PD-1 (Programmed Cell Death 1, PDCD1, CD279, GenBank Accession #NM_005018), dCas9-VP64 and MS2-P65-HSF1 are recruited to the site in the genomic DNA and begin transcription, inducing PD-1 expression. Each pool includes 5 individually validated sgRNA to ensure robust expression.

Application

1. Activate endogenous PD-1 expression in CRISPRa (SAM) cells

Format

5 μ g (0.1 μ g DNA/ μ l)

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.

License Disclosure

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Troubleshooting Guide

For all questions, please email support@bpsbioscience.com.

Validation Data

Gene Target:	Primer ID:	sgRNA Sequence:
PD-1	PD-1-1A	AGGGAGAGAGAGACAGAGAC
PD-1	PD-1-2A	AGGGGGCGGGAGCAAGGGGC
PD-1	PD-1-3A	GGGAGACAGAGGAGATGGGG
PD-1	PD-1-4A	GACAGAGGCAGTGCTGGGGG
PD-1	PD-1-5A	GGGTGAGGAGGGGGTAGGAC

Figure 1. List of sgRNA sequences targeting PD-1

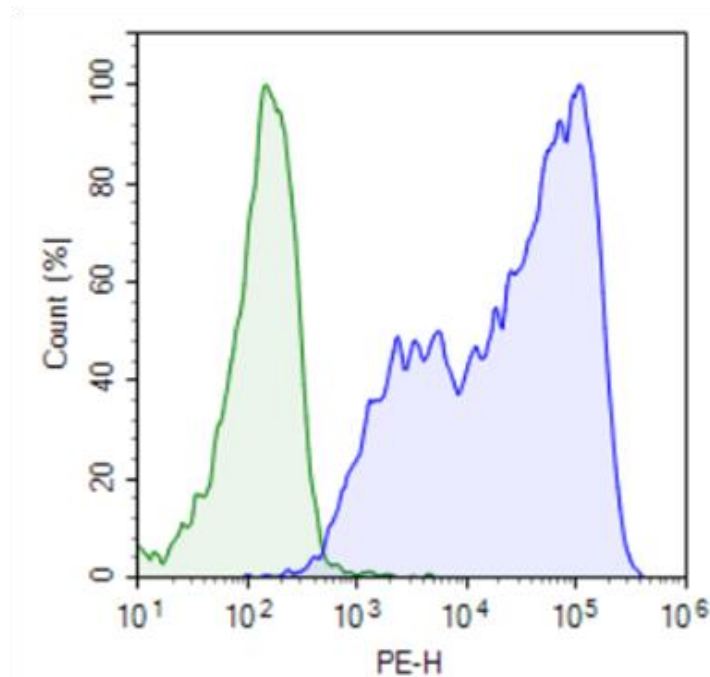


Figure 2. Induction of PD-1 in CRISPRa (SAM) Jurkat cells.

PD-1 sgRNA-MS2 were functionally validated by transfecting into CRISPRa (SAM) Jurkat cells (BPS Bioscience, #78080) and analyzing PD-1 expression by FACS.

CRISPRa (SAM) Jurkat cells (BPS Bioscience, #78080) 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.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
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 (<i>S. pyogenes</i>)	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.