

Product Analysis Certificate

Control Lentiviral CRISPR sgRNA Construct
Non-Targeting Control (NT) in pRSGCCP-U6-sg-CMV-Cas9-2A-Puro (packaged)
Reorder/Cat.# SGCCTL-NT-pRSGCCP-V



Control Lentiviral CRISPR sgRNA Construct

Shipment Contents: Non-Targeting Control (NT) in pRSGCCP-U6-sg-CMV-Cas9-2A-Puro (packaged)
— Store at -80°C

Description:

Cellecta's CRISPR/Cas9 system can be used for CRISPR knockout, CRISPR activation (CRISPRa), or CRISPR interference (CRISPRi) of gene expression *in vivo* or *in vitro* by using a combination of an sgRNA (single guide RNA) along with a Cas9 endonuclease for knockout (e.g. spCas9, saCas9) or catalytically-dead Cas9 for knock-in and repression (e.g. dCas9-VPH and dCas9-KRAB).

The Single-Vector CRISPR system conveniently expresses both sgRNA and Cas9 from the same lentiviral vector. The more flexible Two-Vector system can be used for CRISPR, CRISPRa, or CRISPRi by expressing sgRNA and Cas9 (or dCas9) from different lentiviral vectors. Expression of both the sgRNA and Cas9 (or dCas9) is stable, and the system can be used in dividing or non-dividing cells or whole model organisms.

The Control Lentiviral NT sgRNA Construct expresses a CRISPR sgRNA that does not target any known human, mouse, or rat genes.

The titer of lentiviral packaged constructs provided by Cellecta is functionally determined by transduction of 293T cells and either FACS of RFP- or GFP-positive cells, antibiotic selection assay, or by PCR titering of integrated DNA.

The Two-Vector system requires a separate Cas9- or dCas9-only plasmid, available separately from Cellecta:

<u>Cas9 / dCas9 Expression Plasmids</u>	<u>Plasmid Cat.#</u>	<u>Virus Cat.#</u>
CRISPR Cas9 pRCCH-CMV-Cas9-2A-Hygro	SVC9-PS	SVC9-VS
CRISPR Cas9 pRCCB-CMV-Cas9-2A-Blast	SVC9B-PS	SVC9B-VS
CRISPR Cas9 pRCCG-CMV-Cas9-2A-TagGFP2	SVC9G-PS	SVC9G-VS
CRISPR Cas9 pRCCN-CMV-Cas9-2A-Neo	SVC9N-PS	SVC9N-VS
CRISPR Cas9 pRCCP-CMV-Cas9-2A-Puro	SVC9P-PS	SVC9P-VS
CRISPR Cas9 pRCCR-CMV-Cas9-2A-TagRFP	SVC9R-PS	SVC9R-VS
CRISPRa dCas9 pRDVCCB-CMV-dCas9-VPH-2A-Blast	SVVPHC9B-PS	SVVPHC9B-VS
CRISPRi dCas9 pRDKCCB-CMV-dCas9-KRAB-2A-Blast	SVKRABC9B-PS	SVKRABC9B-VS
CRISPRi dCas9 pRDKCCH-CMV-dCas9-KRAB-2A-Hygro	SVKRABC9H-PS	SVKRABC9H-VS

Biosafety Level: BSL-2
Storage: -80°C
Shelf Life: 1 year from date of receipt
Shipping Conditions: Dry Ice

Product Information (Cellecta Website):

User Manual: <https://www.cellecta.com/resources/product-manuals-and-certificates/>
Vector Map/Sequence: <https://www.cellecta.com/resources/vector-information/>

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Contents:

Catalog # / Reorder #	Description
SGCCTL-NT-pRSGCCP-V	Control Lentiviral CRISPR sgRNA Expression Construct Non-Targeting Control (NT) in pRSGCCP-U6-sg-CMV-Cas9-2A-Puro (packaged) Gene ID: n/a <u>Packaged, >1 x 10⁶ TU:</u> 1.24 × 10⁶ TU , 4.12 × 10 ⁶ TU/ml (300 µl total: 150 µl × 2 vials) Lot# 17030107; Store at -80°C
Target Sequence:	5' -GGCAGTCGTTTCGGTTGATAT-3'
sgRNA Sequence: (gRNA + tracrRNA):	GGCAGTCGTTTCGGTTGATAT GTTTAAAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGT TATCAACTTGAAAAAGTGGCACCCGAGTCGGTGCTTTTTT
Sequencing QC:	NNNTTCTGGGTAGTTTGCAGTTTTAAAAATTATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAA GTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGGCAGTCGTTTCGGTTGATATGTT TAAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC GAGTCGGTGCTTTTTTCGGACTGTAGAACTCTGAACCTCGAGCAATTTAAAAGAAAAGGGGGGATTGGGGGT ACAGTGCAGGGGAAAAGATAGTAGACATAATANCNCNGNNCCATACAA
Sequencing Primer:	5' -ATTAGTACAAAATACGTGACGTAGAA-3' (U6-3)

Structure of sgRNA designed by Cellecta:

5' - 20mer_gRNA_template(target sequence)-tracrRNA -3'

Structure of Target Site (sense or antisense strand):

5' -NNNNNNNNNNNNNNNNNNNNNGG-3' : genomic target + PAM site (sense)
5' -NNNNNNNNNNNNNNNNNNNN-3' : gRNA template, i.e. template DNA / construct insert (sense)
3' -NNNNNNNNNNNNNNNNNNNN-5' : gRNA - RNA expressed from vector (antisense)

Example Genomic Target Site of sg_hPCNA_CO_5 control construct (sense strand):

5' -CCTGGTCCAGGGCTCCATCCTCAAGAAGGTGT-3' : genomic target + PAM site (sense)
5' -CCAGGGCTCCATCCTCAAGA-3' : gRNA template, i.e. template DNA / construct insert (sense)
3' -GGTCCCGAGGTAGGAGTTCT-5' : gRNA - RNA expressed from vector (antisense)

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