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NLS-dCas9-NLS protein

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Background	The functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential in adaptive immunity in select bacteria and archaea. CRISPR uses a Cas9 protein to recognize DNA sequences, with target specificity solely determined by a small guide (sg) RNA and a protospacer adjacent motif (PAM) upon binding to target DNA, the Cas9-sgRNA complex generates a DNA double-stranded break. Based on this RNA- guided nuclease activity, CRISPR has been showed to be a powerful tool in editing the genomes of a broad range of organisms. Furthermore, a repurposed, nuclease-deactivated Cas9 (dCas9) protein has been used to regulate endogenous gene expression and labeling of genomic loci in living and fixed cells.
Size	50 µg
Concentration	1 µg/µl
Source	*E. coli*
Sequence	Mutated CRISPR-associated endonuclease Cas9 (amino acids 1 to 1368) with D10A & H840A (ACCESSION: AKS40378 for Cas9). To facilitate nuclear entry, two nuclear localization signal sequence (NLS) are fused to both N- and C-terminal of dCas9 protein
Appearance	Lyophilized powder.
Formulation	10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 150 mM NaCl, and 50% glycerol (v/v).
Storage and Stability	Recombinant dCas9 (D10A & H840A) protein in solution is temperature sensitive and must been stored at -20°C or below to prevent degradation. Avoid repeated freeze /thaw cycles and keep on ice when not in storage. Stable for 1 year from the date of shipping when stored and handled properly.
Application	Recombinant dCas9 (D10A & H840A) protein is suitable for use in imaging of genomic oci in living cells and fixed cells as well as for gene expression regulation.

Protocol

The protocol listed below is for reference only. The user may optimize the protocol according to their own experiments.

RNP Complex Formation

Gently mix the reaction and incubate at room temperature for 10 minutes. Then place on ice for following transfection by electroporation or liposome, or incubate with permeabilized cells.

Components	Volume	Final Concentration
sgRNA (1000 nM)	1.2 µl	~120 nM
Cas9 Nuclease Protein (1000 nM)	1.2 µl	~120 nM
Opti-MEM	12.6 µl	-
Total	15 µl	

References

1. Deactivated CRISPR Associated Protein 9 for Minor-Allele Enrichment in Cell-Free DNA Amin Aalipour et al. **Clinical Chemistry** 2018 Vol. 64, Issue 2 p307-p916
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5. High-throughput biochemical profiling reveals sequence determinants of dCas9 off-target binding and unbinding Evan A Boyle et al. **PNAS**2017 May, 114 (21) 5461-5466
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