MCLAB
Molecular Cloning Laboratories

320 Harbor Way
South San Francisco, CA 94080
Tel: 650-871-8771
Fax: 650-871-8796
www.mclab.com
mclab@mclab.com

User Manual

Version 1.0

Product name: dCas12a

Cat #: dCAS12A-100

Description:

The dCas12a is a mutant version of the Cas12a protein. Unlike wild-type Cas12a, it can bind to target DNA without cleaving it, making it an ideal tool for applications requiring specific DNA targeting, such as transcription regulation, epigenetic modification, or DNA labeling. Additionally, biotinylated dCas12a includes an N-terminal biotin tag, enabling efficient use in streptavidin-based experiments for DNA-protein interaction studies and high-throughput screening.

Protocols:

Applications and Sample Protocol Overview

A. DNA Binding Assay

1. Design crRNA specific to your target DNA.
2. Assemble dCas12a-crRNA complex:
    - Mix dCas12a (1 µg) with crRNA (final concentration: 100 nM).
    - Incubate at 37°C for 15 minutes.
3. Introduce the complex into your experimental setup, such as live cells or lysates.

B. Streptavidin-Based Pull-Down Experiment

1. Prepare DNA sample and biotinylated dCas12a.
2. Incubate DNA sample with dCas12a in binding buffer for 30 minutes at room temperature.
3. Add streptavidin-coated beads and incubate for 1 hour at 4°C with gentle rotation.
4. Wash beads 3 times with wash buffer.
5. Elute bound complexes for downstream analysis.

C. Transcriptional Regulation

1. Fuse dCas12a with transcriptional activators or repressors (e.g., VP64, KRAB).
2. Use guide crRNA to target specific promoters or enhancers.
3. Monitor changes in gene expression using RT-qPCR or reporter assays.

D. Epigenetic Modification

1. Conjugate dCas12a with epigenetic effectors (e.g., DNA methyltransferase, histone acetylase).
2. Target specific genomic loci for modification.

Troubleshooting:

Problem	Possible Cause	Solution
Weak DNA binding signal	Insufficient dCas12a-crRNA complex formation	Optimize crRNA concentration or sequence.
Loss of activity	Improper storage conditions	Use freshly thawed aliquots. Avoid freeze-thaw.
High background in assays	Non-specific binding of biotin tag	Add blocking steps with streptavidin.
Low yield in pull-down	Insufficient incubation with beads	Increase incubation time or bead concentration.

References:

1. Abudayyeh, O. O., Gootenberg, J. S., Essletzbichler, P., Han, S., Joung, J., Belanto, J. J., ... & Zhang, F. (2017). RNA targeting with CRISPR-Cas13. Nature, 550(7675), 280–284. https://doi.org/10.1038/nature24049
2. Cox, D. B. T., Gootenberg, J. S., Abudayyeh, O. O., Franklin, B., Kellner, M. J., Joung, J., & Zhang, F. (2017). RNA editing with CRISPR-Cas13. Science, 358(6366), 1019–1027. https://doi.org/10.1126/science.aaq0180
3. Wessels, H. H., Méndez-Mancilla, A., Guo, X., Legut, M., Daniloski, Z., & Sanjana, N. E. (2020). Massively parallel Cas13 screens reveal principles for guide RNA design. Nature Biotechnology, 38(6), 722–727. https://doi.org/10.1038/s41587-020-0456-9