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User Manual

Version 1.0

Product name: dCas13a

Cat #: dCas13a-100, dCas13a-200, dCas13a-300

Description:

The dCas13a is a mutant version of the Cas13a protein. Unlike wild-type Cas13a, it can bind to target RNA without cleaving it, making it ideal for applications where RNA localization, capture, or stabilization is required. Additionally, biotinylated dCas13a includes an N-terminal biotin tag, enabling efficient use in streptavidin-based experiments for RNA-protein interaction studies, RNA localization assays, and high-throughput screening.

Protocols:

Applications and Sample Protocol Overview

A. RNA Binding Assay

1. Design RNA guide specific to your target RNA.
2. Assemble dCas13a-guide RNA complex:
    - Mix dCas13a (1 µg) with guide RNA (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 RNA sample and biotinylated dCas13a.
2. Incubate RNA sample with dCas13a 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.

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C. RNA Imaging

1. Conjugate dCas13a with fluorophores using a streptavidin-fluorophore complex.
2. Use in live or fixed cells for RNA localization studies.

Troubleshooting:

Problem	Possible Cause	Solution
Weak RNA binding signal	Insufficient dCas13a-guide complex formation	Optimize guide RNA 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:

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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