

## Product Information

### dCas9-3xFLAG™-Biotin Protein

Cas9 (D10A, H840A) - NLS-3xFLAG™-Biotin  
from *Streptococcus pyogenes*  
expressed in *Escherichia coli*

Catalog Number **DCAS9PROT**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

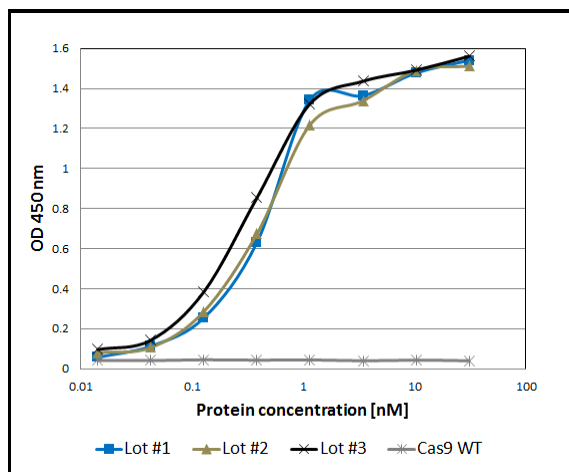
## TECHNICAL BULLETIN

### Product Description

dCas9-3xFLAG-Biotin is a recombinant catalytically dead Cas9 (dCas9) protein derived from a type II-A *Streptococcus pyogenes* Cas9 and expressed in *E. coli*. The dCas9 protein carries D10A and H840A mutations, which inactivate the nuclease domains without compromising target DNA binding. At the C-terminus, the protein is tagged with an SV40 large T antigen nuclear localization signal (NLS), 3xFLAG epitope, and biotin for ANTI-FLAG®-based or streptavidin-based pull-down and molecular detection (see Figure 1). Therefore, the dCas9 protein can be used for a variety of applications including targeted DNA isolation and detection.<sup>1,2,3</sup>

### Figure 1.

Sandwich ELISA of dCas9-3xFLAG-Biotin.

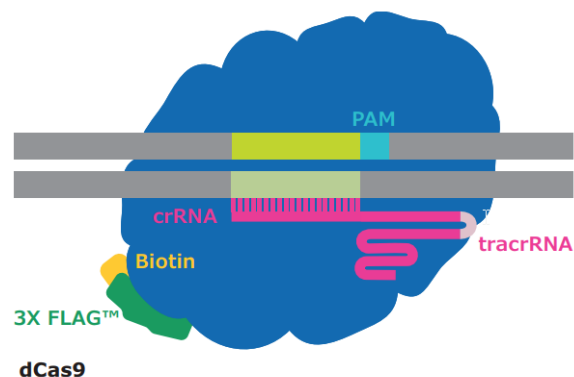


A plate coated with ANTI-FLAG antibody (Catalog number P2983) was incubated with dCas9-3xFLAG-Biotin (Lot#1, 2, and 3) or non-biotinylated Cas9 Protein (Cas9 WT, Catalog number CAS9PROT), washed and then incubated with ExtrAvidin®-HRP (Catalog number E2886) and color developed with TMB (Catalog number T4444).

The protein can be combined with SygRNA™ synthetic crRNA and tracrRNA to form a three-component RNP complex to bind a target DNA of interest (see Figure 2). Alternatively, the protein can be complexed with an *in vitro* transcribed (IVT) single guide RNA (sgRNA) for target DNA binding.

### Figure 2.

Target recognition by dCas9 RNP complex.



The dCas9 ribonucleoprotein is made up of the dCas9 protein and a guide RNA, which can be delivered as a single guide RNA or as a tracrRNA and crRNA. The 5' portion of the crRNA is variable and complementary to the target of interest, while the tracrRNA sequence is constant.

## Components

dCas9-3xFLAG-Biotin is provided lyophilized with a Reconstitution Solution and a Dilution Buffer.

- One vial of lyophilized dCas9-3xFLAG-Biotin protein, Catalog Number D110010, 50 µg or 250 µg
- Reconstitution Solution, Catalog Number RSOLUTION, 1 mL of 50% glycerol in water
- Dilution Buffer, Catalog Number DBUFFER, 1 mL of 20 mM HEPES, pH 7.5, with 200 mM NaCl.

## Reagents Required but Not Provided.

- Custom SygRNA (synthetic crRNA) can be ordered via this [link](#). Alternatively, to purchase custom crRNAs click "Request Quotation for Custom crRNA Synthesis" at [sigma.com/SygRNA](http://sigma.com/SygRNA). Enter the 20 bp genomic DNA target sequence (5' to 3') plus the adjacent 3' PAM site.
- tracrRNA - Catalog Number TRACRRNA05N
- Water, PCR Reagent grade - Catalog Number W1754

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Preparation Instructions

1. Resuspend the lyophilized dCas9 protein with the supplied Reconstitution Solution (Catalog Number RSOLUTION).
  - a. For 250 µg vials, add 50 µL of Reconstitution Solution per vial to achieve a concentration of ~5 mg/mL (25 pmol/µL).
  - b. For 50 µg vials, add 30 µL of Reconstitution Solution per vial to achieve a concentration of ~1.7 mg/mL (8 pmol/µL).

Note: Precise quantities vary from lot-to-lot, please refer to the certificate of analysis for exact protein content per vial.

2. Gently tap tube to completely dissolve lyophilized powder, incubate for 10 minutes on ice, and spin tube briefly to bring material to bottom of tube.
3. If a lower concentration of dCas9 protein is required, dilute the dCas9 protein solution with the supplied Dilution Buffer (Catalog Number DBUFFER) immediately before use. Store diluted protein on ice, up to 6 hours.

## Storage/Stability

All components are shipped at ambient temperature. Store all components at –20 °C upon arrival. Once resuspended in the provided Reconstitution Solution, the dCas9 protein solution should be stored at –20 °C.

## Procedure

### RNP Preparation Recommendation

- Assemble guide RNA:dCas9 RNP complexes on ice, immediately before use.
- In all instances, combine equal molar amounts of crRNA:tracrRNA.
- Preparing RNP in a molar ratio ranging from 1:1:1 to 5:5:1 (crRNA:tracrRNA:dCas9 protein) is suggested. Further optimization may be required for certain targets.

### Prepare RNP complexes

This step may be performed before or in parallel to protein preparation.

1. Dilute SygRNA crRNA and tracrRNA (or IVT sgRNA) to a 20 µM working solution using a 10 mM Tris buffer, pH between 7 and 8.
2. Pipette 3 to 6 µL (60 to 120 pmol) of each RNA to a sterile microcentrifuge tube on ice.
3. Optional: Anneal the crRNA and tracrRNA by incubating the mixture for 5 minutes at 95 °C, then placing the mixture on ice for 20 minutes.
4. Add 20 to 60 pmol of dCas9 protein to tube containing synthetic crRNA and tracrRNA. Gently pipette up-and-down to mix.
5. Incubate at room temperature for 5-10 minutes for complex formation.

## References

1. Guk, K. et al., A facile, rapid and sensitive detection of MRSA using a CRISPR-mediated DNA FISH method, antibody-like dCas9/sgRNA complex. *Biosensors and Bioelectronics*, **95**, 67-71 (2017).
2. Aalipour, A. et al., Deactivated CRISPR Associated Protein 9 for Minor-Allele Enrichment in Cell-Free DNA. *Clinical Chemistry*, **64**, 307-16 (2018).
3. Deng, W. et al., CASFISH: CRISPR/Cas9-mediated *in situ* labeling of genomic loci in fixed cells. *Proceedings of the National Academy of Sciences*, **112**, 11870-5 (2015).

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## Troubleshooting Guide

Suspected Issue	Solution
dCas9 protein has denatured after long term storage in Dilution Buffer.	Dilution of the dCas9 protein in the provided dilution buffer is only recommended for immediate use. For long term storage, keep the protein lyophilized or resuspended in the provided Reconstitution Solution and stored at $-20^{\circ}\text{C}$ .
crRNAs and tracrRNAs need to be annealed before complexing with dCas9 protein.	Anneal the crRNA and tracrRNA by mixing them in the desired ratio, incubating the mixture for 5 minutes at $95^{\circ}\text{C}$ followed by 20 minutes on ice.
crRNAs and tracrRNAs are degraded.	For optimal performance, only quality-verified guide RNA should be used. Use RNase free pipette tips, preferably those having an aerosol barrier.

## US Patents Pending

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