

SpCas9 Nuclease (Lyophilized)

#V32101E / V32101T / V32101S

Version e155.1.0

■ Introduction

SpCas9 is a DNA endonuclease guided by crRNA and tracrRNA (or sgRNA alone), which is derived from *S. pyogenes*. SpCas9 specifically recognizes and cleaves dsDNA target in a PAM (NGG)-dependent manner. The cleavage site of Cas9 in the target sequence is 3 bps away from the PAM site. In addition, SpCas9 can also specifically cleave ssDNA or ssRNA in the presence of DNA PAMmer sequences (for details, please refer to PMID: 24476820; PMID: 25274302). SpCas9 can also be used in *in vitro* experiments for cleaving target DNA and cloning target fragments (for details, please refer to PMID: 26323354).

This product is lyophilized glycerol-free SpCas9 nuclease, which can be shipped and stored at room temperature. After being dissolved with the Reconstitution Solution, it shows the same performance as the non-lyophilized type of SpCas12b Nuclease (#32101, ToloBio). This product can be provided in a variety of lyophilized forms such as lyophilized powder and lyophilized beads to meet the needs of different downstream applications.

■ Components

SpCas9 Powder	V32101E-SP	V32101E-01	V32101E-02
○ SpCas9 Lyophilized Powder (SCREW CAP TUBES)	10 µg	100 µg	100 µg × 5
● Reconstitution Solution	200 µL	200 µL	1 mL
10 × HOLMES Buffer for Cas9	1 mL	1 mL	1 mL × 5
● <i>It is recommended to use 124 µL of reconstitution solution to reconstitute 100 µg SpCas9 lyophilized powder, generating 5 µM (5 pmol/µL) liquid enzyme. Once dissolved, the liquid enzyme should be stored at -20 °C or below.</i>			
SpCas9 Powder (Single Reaction)	V32101T-SP	V32101T-01	V32101T-02
● SpCas9 Lyophilized Powder (8-TUBE STRIPS)	8 T	48 T	96 T
10 × HOLMES Buffer for Cas9	1 mL	1 mL	1 mL
● <i>The amount of SpCas9 for a single reaction is 5 pmol and is recommended for a 20 µL CRISPR reaction system.</i>			
SpCas9 Beads (Single Reaction)	V32101S-SP	V32101S-01	V32101S-02
● SpCas9 Lyophilized Beads (8-TUBE STRIPS)	8 T	48 T	96 T
10 × HOLMES Buffer for Cas9	1 mL	1 mL	1 mL
● <i>The amount of SpCas9 in a single lyophilized bead is 5 pmol and is recommended for a 20 µL CRISPR reaction system.</i>			

■ Storage

This product is stored and shipped at room temperature. After reconstitution, the liquid enzyme should be stored at

-20 °C or below and is stable for at least one month. Repeated freezing and thawing should be avoided for reconstituted liquid enzyme.

■ Experimental Procedure

SpCas9 (Cat # V32101E) CRISPR *Cis*-cleavage Experiment

1) Prepare the reaction mixture of CRISPR *Cis*-cleavage Experiment on ice as below.

Components	Volume	Final Concentration
10 × HOLMES Buffer for Cas9 (#32041)	2 μL	1 ×
5 μM SpCas9 Nuclease	1 μL	250 nM
10 μM sgRNA	0.5 μL	250 nM
1 μM Target dsDNA	0.5 μL	25 nM
Nuclease-free Water	Up to 20 μL	

2) Put the reaction mixture at 37 °C for 30 min and then inactivate the enzyme at 85 °C for 5 min . Analyze products using nucleic acid electrophoresis.

SpCas9 (Cat# V32101T/V32101S) CRISPR *Cis*-cleavage Experiment

1) Prepare the reaction mixture of CRISPR *Cis*-cleavage Experiment on ice as below.

Components	Volume	Final Concentration
10 × HOLMES Buffer for Cas9 (#32041)	2 μL	1 ×
10 μM sgRNA	0.5 μL	250 nM
1 μM Target dsDNA	0.5 μL	25 nM
Nuclease-free Water	Up to 20 μL	

2) Dissolve the lyophilized powder/bead for a single reaction using the 20 μL reaction mixture prepared above.

3) Put the reaction mixture at 37 °C for 30 min and then inactivate the enzyme at 85 °C for 5 min . Analyze products using nucleic acid electrophoresis.

■ Experimental Results

