AccuTool™ Cas9 recombinant protein (V0/2021-07-14)

[Cat. No.] Please refer to the **Ordering Information**

Introduction

RNA-Guided Endonucleases (RGENs) are ready-to-use Cas9 recombinant proteins for your genome editing experiments. *AccuTool*™ Cas9 recombinant proteins are recombinant *Streptococcus pyogenes* Cas9 (WT) protein, purified from *E. coli*. The Cas9 recombinant proteins and custom-designed guide RNA (aRGENs) form a stable ribonucleoprotein (RNP) complex, which can be used on cells that are difficult to be transfected like primary cells. The Cas9 Nickase (D10A) generates a DNA nick rather than a double-strand breaks (DSBs), and the Cas9 Dead (D10A/H840A) has no endonuclease activity but remains its ability to bind onto the target sequences. Sniper Cas9 improves specificity and on-target activity by reducing off-targeting. The reagents can be used for *in vitro* and *in vivo* without additional processing.

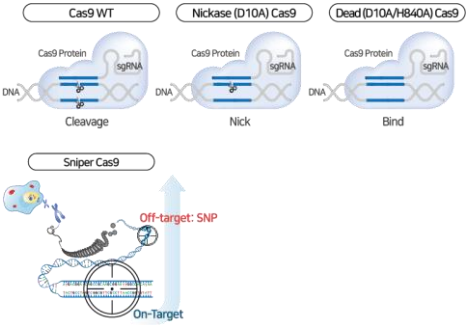


Figure 1. Cas9 recombinant proteins

Applications

- Genome editing
- Drug discovery: CRISPR library screening, target validation
- Bioprocessing: Cell line engineering
- Agriculture: Plant breeding

Components

Components	Amount
Recombinant SpCas9 (WT or Nickase or Dead or Sniper) protein	50 µg
10X Reaction buffer	1 ml
1X Dilution buffer	1 ml

*** Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Specifications

- *AccuTool*™ Cas9 recombinant proteins are recombinant *Streptococcus pyogenes* Cas9 (WT) protein, purified from *E. coli*.
- *AccuTool*™ Cas9 recombinant proteins concentration: 1 mg/ml.

Buffer Composition

10X Reaction buffer	1 M NaCl, 500 mM Tris-HCl, 100 mM $MgCl_2$, 1 mg/ml BSA, pH 7.9

Storage Buffer

Cas9 recombinant protein is supplied in 10 mM Tris-HCl, 300 mM NaCl, 0.1 M EDTA, 1 mM DTT, 50% glycerol, and stabilizer, pH 7.4.

Quality Assurance

- Protein purity: >95% by SDS-PAGE with Coomassie Blue Staining.
- RNase Activity: No
- DNase Activity: No
- Protease Activity: No

Storage

- *AccuTool*™ Cas9 recombinant proteins 50 µg (1 mg/ml)/vial are delivered at -20°C.
- Store the appropriate aliquot of proteins at or below -70°C. Do not store in a frost-free freezer.

Online Resources

Korean English

Visit our **product page** for additional information and protocols.

Ordering Information

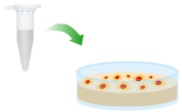
Description		Cat. No.
AccuTool™ Recombinant SpCas9 WT protein	50 µg	ATS-0010
	50 µg x 2 ea	ATS-0011
	50 µg x 5 ea	ATS-0012
AccuTool™ Recombinant SpCas9 Nickase (D10A) protein	50 µg	ATS-0016
	50 µg x 2 ea	ATS-0017
	50 µg x 5 ea	ATS-0018
AccuTool™ Recombinant SpCas9 dead (D10A/H840A) protein	50 µg	ATS-0019
	50 µg x 2 ea	ATS-0020
	50 µg x 5 ea	ATS-0021
AccuTool™ Recombinant Sniper Cas9 protein	50 µg	ATS-0025
	50 µg x 2 ea	ATS-0026
	50 µg x 5 ea	ATS-0027

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements, and other changes to its products, services, specifications, or product descriptions at any time without notice.

Explanation of Symbols

Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td colspan="3">Digestion of target sequence in vitro using CRISPR sgRNAs</td></tr>
<tr><td>1</td><td> 
Preparation of reaction mixture</td><td>1. Set up the reaction mixture as below.
<table><tr><th>Components</th><th>Amount</th><th></th></tr><tr><td>Cas9 Protein</td><td>500 ng</td><td>(100-1,000 ng)</td></tr><tr><td>CRISPR sgRNA</td><td>250 ng</td><td>(100-700 ng)</td></tr><tr><td>Targeting substrate</td><td>100-150 ng</td><td>PCR product</td></tr><tr><td></td><td>80 ng</td><td>(Plasmid)</td></tr><tr><td>10X Reaction buffer</td><td>1 µl</td><td></td></tr><tr><td>D.W.</td><td>To 10 µl</td><td></td></tr></table></td></tr>
<tr><td>2</td><td> 
Incubation of reaction mixture</td><td>2. Incubate the reaction mixture at 37°C for 1 hr.
3. Add 4 µg of RNase and incubate for 15 min at 37°C.
4. Add 1 µl of STOP solution to the reaction mixture and incubate for 15 min at 37°C.
[STOP solution: 30% glycerol, 1.2% SDS, 250 mM EDTA (pH 8.0)]
5. Analyze on 2% agarose gel.</td></tr>
<tr><td colspan="3">General guidelines for the application of CRISPR RNP to cultured cells by lipid-based transfection (Lipofection)
• The amounts of the reagents given in the protocol below are for one well of a 24-well plate. For other reaction formats, scale the amounts of reagents up or down accordingly.</td></tr>
<tr><td>1</td><td> 
Preparation of transfection reagent</td><td>1. Add Cas9 RNP complex (0.5 µg of Cas9 Nuclease and 250 ng of CRISPR sgRNA) to 50 µl Opti-MEM I Reduce Serum Medium.
2. In a separate tube, dilute the transfection reagent by adding 4 µl of the Lipofectamine 2000 transfection reagent to 50 µl of Opti-MEM I Reduce Serum Medium and mix gently.
3. Incubate for 5 min at room temperature.
4. Add the diluted transfection reagent to the tube containing Cas9 protein/gRNA RNP complexes and mix gently.
5. Incubate at room temperature for 20 min to allow the formation of Cas9/gRNA-lipid complexes.</td></tr>
<tr><td>2</td><td> 
Cell transfection</td><td>6. Add the Cas9/gRNA-lipid complexes to the 1x10^5 NIH3T3 cells to be transfected. Swirl the plates gently to allow the mixing of the transfection mixture with the medium.
7. Incubate the plate at 37°C in a humidified CO_2 incubator in a cell culture incubator for 2-3 days, and proceed with sample assay to determine the genome editing efficiency by T7E1 assay or targeted deep sequencing.</td></tr>
<tr><td></td><td> 
Option</td><td>Refer to the electroporation method for highly efficient transfection only.</td></tr>
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