Datasheet for ABIN3071565

CRISPR-Cas9 (Active) protein (NLS)

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Overview

Quantity:	50 µg
Target:	CRISPR-Cas9
Origin:	Streptococcus pyogenes
Source:	Escherichia coli (E. coli)
Protein Type:	Recombinant
Biological Activity:	Active
Purification tag / Conjugate:	NLS
Application:	In vitro Cleavage Assay (IVCA), In vivo Gene Editing (IVGE), Microinjection (MI), RNA Electroporation (REP), Transfection (T)

Product Details

Specificity:	Activity test Cas9 site-specific digestion: We used in vitro digestion of a linearized plasmid to determine the activity of the Cas9 nuclease. It is a sensitive assay for GenCrispr Cas9 quality control. The linearized plasmid containing the target site: (CATCATTGGAAAACGTTCTT) can be digested with gRNA: (CAUCAUUGGAAAACGUUCUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUUU) and GenCrispr Cas9. Two cleavage DNA fragments (812 bp and 1898 bp) are determined by agarose gel electrophoresis. A 20 µL reaction in 1xCas9 Nuclease Reaction Buffer containing

Product Details

	160 ng linearized plasmid, 40 nM gRNA and 20 nM GenCrispr Cas9 for 2 hour at 37 °C results in 90 % digestion of linearized plasmid as determined by agarose gel electrophoresis.
Characteristics:	GenCrispr Cas9-C-NLS is produced by expression in an E. coli strain carrying a plasmid encoding the Cas9 gene from Streptococcus pyogenes with a C terminal nuclear localization signal (NLS).
Purification:	purified
Purity:	> 95 % pure as determined by SDS-PAGE with Coomassie Blue detection.
Endotoxin Level:	Endotoxin level is <0.1eu/ug test by gel-clot method: limit test
Components:	GenCrispr Cas9-C-NLS 10X Reaction Buffer

Target Details

Target:	CRISPR-Cas9
Alternative Name:	Cas9-C-NLS Nuclease
Background:	Cas9 nuclease is an RNA-guided endonuclease that can catalyze cleavage of double stranded DNA. This kind of targeted nuclease is a powerful tool for genome editing with high precision. Cas9 protein forms a very stable ribonucleoprotein (RNP) complex with the guide RNA (gRNA) component of the CRISPR/Cas9 system. The Cas9 RNP complex can localize to the nucleus immediately upon entering the cell with the addition of a nuclear localization signal (NLS). There is no requirement for transcription and translation compared with mRNA or plasmid systems. Additionally, the Cas9 RNP complex is rapidly cleared from the cell minimizing the chance of off-target cleavage when compared to other systems (Kim, et al. 2014). This DNA-free system avoids the risk of inserting foreign DNA into the genome, which can be quite useful for gene editing-based disease therapy. GenScript has developed a Cas9-C-NLS nuclease which contains a nuclear localization sequence (NLS) on the C-terminus of the protein to meet all the researchers' requirements (e.g. in vitro cleavage assay, RNP complex transfection, and micro injection).

Application Details

Application Notes:	Screening the highly efficient and specific targeting gRNAs using in vitro DNA cleavage. In vivo gene editing combined with specific gRNA by electroporation or injection.
Comment:	1. DNA-free: no external DNA added to system.

Application Details

	2. High cleavage efficiency: NLS ensures the entry of Cas9 protein into nuclei. 3. Low off target: transient expression of Cas9 nuclease. 4. Time-saving: no need for transcription and translation.
Restrictions:	For Research Use only

Handling

Concentration:	1 mg/mL
Buffer:	10X Reaction Buffer: 200 mM HEPES, 1M NaCl, 50 mM MgCl2, 1 mM EDTA, pH 6.5 at 25 °C. 1X Storage Buffer: 20 mM Tris, 600 mM NaCl, 0.1 mM EDTA,1 mM DTT,50 % Glycerol PH 7.4 at 25 °C)
Preservative:	Dithiothreitol (DTT)
Precaution of Use:	This product contains Dithiothreitol (DTT): a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	GenCrispr Cas9-C-NLS nuclease is supplied with 1X storage buffer (20 mM Tris, 600 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol PH 7.4 at 25°C) and recommended to be stored at -20°C. Diluent Compatibility: Diluent Buffer: 300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 at 25°C).