Tinzyme Co., Limited

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Cas9 NLS

Product Number: CAS09N

Shipping and Storage

Store at -30 ~ -15℃ and transport at ≤ 0℃.

Components

Component	CAS09N 500pmol	CAS09N 1000pmol
Cas9 NLS (20μM)	25μl	50μl
Cas9 NLS Reaction Buffer (10×)	1ml	2ml

Description

CRISPR/Cas9 is an adaptive immune defense system formed by bacteria and archaea during their long-term evolution.The CRISPR/Cas9 system integrates fragments of invading phage and plasmid DNA into the CRISPR sequence and utilizes corresponding CRISPR RNAs (crRNAs) to guide the degradation of homologous sequences by Cas9 proteins, thereby providing immunity.The artificially modified Cas9/sgRNA system uses sgRNA (short guide RNA) to guide Cas9 proteins to recognize and cleave double stranded DNA with sgRNA targets, which can be used for gene knockout and precise DNA editing operations.The Cas9 endonuclease provided by this product allows the protein to enter the nucleus for genome editing by adding a nuclear localization signal (NLS) at the N-terminus of the protein. This product is a pure protein system to avoid plasmid or virus interference during CRISPR gene knockout.

Source

Obtained through E.coli recombination, expression, and purification, the expressed gene is derived from Streptococcus pyogenes.

Application

Cell gene editing, in vitro screening of efficient gRNA sequences, specific double stranded DNA cleavage guided by gRNA, selective linearization of double stranded DNA containing specific sequences, etc.

Unit definition

1 unit refers to the amount of enzyme required to add 0.5pmol of dNTP to acid insoluble precipitate during a 10 minute reaction at 30℃.

Protocol

1. Prepare the system reaction solution (cis shear experiment) according to the following suggestions:

Reagent	Volume
Nuclease-free ddH_2O	20 μl
Cas9 NLS Reaction Buffer (10×)	3 μl
SgRNA	3 μl
Cas9 NLS	1 μl
Total	27 μl

Note:1)To ensure the highest cutting efficiency, the molar ratio of Cas9 Nuclease and sgRNA to target DNA should be at least 10:10:1 or higher.

2)Generally, use 30μl system , but it can also be scaled up in equal proportions.

3)Please dilute sgRNA to 300nM using Nuclease free ddH_2O before the experiment.

4)Please wear a mask and use Nuclease free consumables and reagents to avoid the degradation of sgRNA during the experiment.

2. Incubate at 37℃ for 10 minutes.
3. Add 3μl of 30nM DNA.

Note:Dilute DNA to 30nM using Nuclease free ddH_2O.

4. Shake well and centrifuge briefly for collection.
5. Incubate at 37℃ for 1 hour.
6. The reaction products can be directly analyzed by agarose gel electrophoresis.

Note: If electrophoresis is not done immediately, EDTA can be added to terminate the reaction.

Note

1. When using this product, it will involve the operation of gRNA and DNA, and attention must be paid to the relevant operations of RNase free and DNase free.All self prepared reagents and consumables should also be Nuclease free.If there may be nuclease contamination, consider treating overnight with 0.01% DEPC and then using it after high-temperature and high-pressure treatment. It is recommended to wear a disposable mask during operation.
2. This product is for scientific research purposes only and shall not be used for clinical medical diagnosis or other unreasonable purposes.