Product Analysis Certificate

PRODUCT	**Purified Cas9-EGFP protein (NLS-Cas9-EGFP)**
CATALOG #	**CAS420A-1**
LOT #	**C21061906**
STORAGE	**-20°C (long term)**
SHELF LIFE	**12 months from date of receipt with proper storage**
SHIPPING	**Blue ice**

DESCRIPTION

NLS-Cas9-EGFP is a fusion protein, which contains a nuclear localization signal (NLS) on its N terminal end, and an EGFP and a 6x(His) sequence on the C terminal end. 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 highly stable ribonucleoprotein (RNP) complex with the guide RNA (gRNA) component of the CRISPR/Cas9 system. When Cas9 is expressed with an NLS sequence, the Cas9 RNP complex can localize to the nucleus immediately upon entering the cell. There is no requirement for *in vivo* transcription or translation, which improves the efficiency of this method over plasmid-based systems. Additionally, the Cas9 RNP complex is rapidly cleared from the cells minimizing the chance of off-target cleavage when compared to other systems (Kim, et al. 2014). The EGFP tag can be used as a reporter for tracking or sorting transfected cells, which enables enrichment of cell populations for desired genome edits via fluorescence activated cell sorting (FACS). It significantly reduces the labor and cost associated with single cell cloning and genotyping in genome editing applications.

PRODUCT SOURCE

NLS-Cas9-EGFP is produced by expression from an *E. coli* strain.

KEY FEATURES:

- DNA-free: no external DNA added to system
- High cleavage efficiency: NLS ensures the efficient entry of the Cas9 protein into nuclei
- Low off target effects: transient expression of Cas9 nuclease improves specificity of cleavage
- Time-saving: no need for transcription and translation
- Reduced labor: enrich cell populations for desired genome edits via EGFP-based FACS. The C-terminal His-tag increases the choice of detection methods for the fusion protein..

PACKAGE CONTENTS

Cat#	Description	Amount
CAS420A-1	Purified Cas9-EGFP protein (NLS-Cas9-EGFP) (3 µg/µl)	**50 µg/vial**
10X Reaction Buffer	200 mM HEPES, 1 M NaCl, 50 mM MgCl2, 1 mM EDTA, pH 6.5 at 25°C	**500 µl**

Note: 1000 nM is equal to 190 ng/µl.

HANDLING GUIDELINES

- Store product at -20°C for long term

QC RESULTS

Test Items	Specifications	Results
Purity	>95%	Qualified
Qualified Bioactivity	>90% (in vitro) cleavage efficiencies	Qualified
DNase activity	No DNase activity	Qualified
RNase activity	No RNase activity	Qualified

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).

PROTOCOLS:

IN VITRO CLEAVAGE ASSAY:

1. Set up the reaction mixture as below. Add Cas9-EGFP protein and sgRNA the last for best activity.

Components	Amount
Cas9-EGFP protein	20 nM
sgRNA	40 nM
Target DNA	95 ng PCR product
	or 160 ng plasmid
10x reaction buffer	1 µl
Nuclease free water	Up to 20 µl

2. Incubate the reaction mixture at 37 °C for 1-2 hr.
3. Analyze on agarose gel.

VALIDATION DATA:

1.NLS-Cas9-EGFP site-specific digestion:

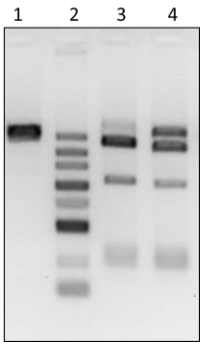

Fig 1: In vitro DNA cleavage assay with NLS-Cas9-EGFP nuclease Reactions were set up according to recommended conditions, and cleavage products were resolved on a 1% agarose gel. Input DNA is EcoR V-linearized pUC57 plasmid DNA. Lane 1, DNA + gRNA; lane 2, marker; lane 3 and 4, DNA + gRNA + NLS-Cas9-EGFP 100 ng (lane 3) or 50 ng (lane 4)

2. Cell Transfection and Gene Editing Efficiency Testing

To test the transfection efficiency and gene editing efficiency of NLS-Cas9-EGFP, 20 µg NLS-Cas9-EGFP and 10 µg control gRNA were introduced into 293T cells by electrophoresis, which lead to over 90% transfection rate. Compared to NLS-Cas9 positive control, NLS-Cas9-EGFP caused a similar gene editing efficiency.

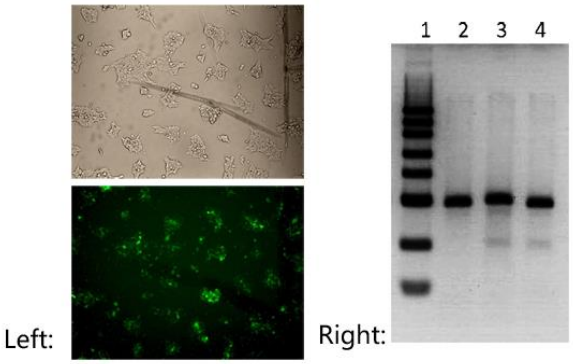

Fig 2: Left: Cell transfection assay. 12 h after electroporation, cells were observed under bright or fluorescence microscope. Right: in vivo gene editing efficiency assay by T7E1. Lane 1, marker; lane 2, negative control; lane 3, gRNA + NLSCas9-EGFP; lane 4, gRNA + NLS-Cas9

Important Licensing Information

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