LD Biopharma, Inc.
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- PRODUCT DATA SHEET -

Name of Product: Recombinant Cas9-NTS Protein
Catalog Number: bRP-1510-7
Manufacturer: LD Biopharma, Inc.

Introduction

CRISPR-Cas9 systems have shown tremendous promise as heterologous tools for genome editing and transcriptional regulation. CRISPR (clustered regularly interspaced short palindromic repeat) is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. Recent data indicated that by intracellular delivery Cas9 protein, which carry nuclei translocation signal (NTS) domain either in DNA transfection or protein-delivery with gRNA, this protein could be used for various mammalian cell genome editing, and site-specific gene transcription regulation.

Full-length bacterial Cas9 (1367 aa) gene was constructed using full-length gene synthesis strategy. His tag was added at N-terminal of Cas9 protein, and nuclei translocation signal (NTS) was inserted at C-terminal. This protein was expressed in E.coli as soluble protein (163.7 KD). The final product was purified with two step column chromatography.

Gene Symbol:	Cas9 (RISPR-associated endonuclease Cas9/Csn1; SpyCas9)
ccession Number:	Uniprot: Q99ZW2
Species:	Streptococcus pyogenes serotype M1
Size:	20µg / Vial
Composition:	0.4 mg/ml, sterile-filtered, in 50 mM Tri-HCl (pH 8.0), with 400 mM NaCl and 20% Glycerol.

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Storage: In Liquid. Keep at -80°C for long term storage. Product is stable at 4 °C for at least 15 days.

Key References

Sojung Kim, et al. *Highly efficient RNA-guided genome editing in human cells via delivery of purified CAs9 ribonucleoproteins*. Genome Res. Aril 8, on line publication. (2014).

Suresh Ramakrishna, et al. *Gene disruption by cell-penetrating peptide-mediated delivery of Cas9 protein and guide RNA*. Genome Res. Aril 2, on line publication. (2014).

John A Zuris, et al. *Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo*. Nature Biotechnology. published online 30 October doi: 10. 1038 / nbt.3081. (2014)

Chen Yu, et al. *Small Molecules Enhance CRISPR Genome Editing Pluripotent Stem Cells*. Cell Stem Cell.16, 142-147. (2015)

Cong L, et al. *Multiplex genome engineering using CRISPR/Cas systems*. Science 339(6121): 819-823. (2013)

Horvath P, Barrangou R. *CRISPR/Cas, the immune system of bacteria and archaea*. Science 327(5962): 167-170. (2010)

Applications

1. May be used for in vitro Cas9 mediated mammalian cell gene editing study by intracellular delivery this protein with target gene specific gRNA either together as complex or separagely with “ProFectin” reagent.

2. Highly purified Cas9 antigen, may be used for specific antibody production.

Quality Control

1. Purity: > 96% by SDS-PAGE.

2. Endotoxin level: < 1 EU/ug.

Recombinant Protein Sequence

MKHHHHHHQEFRSDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALL
FDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH

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PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSD
VDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIAL
SLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRV
NTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN
REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL
KEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFED
REMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKP
ENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGR
DMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWR
QLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGD
YKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDK
GRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVA
YSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF
ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDE
IIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTID
RKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGLYETRIDLSQLGGDSRADPKKKRKV