OriGene Cas9 Nuclease
Cat. No. TP790148

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Description

Wild type *Streptococcus pyogenes* Cas9 protein is an RNA-guided endonuclease that can be used for the site-specific cleavage of double stranded DNA. OriGene Cas9 nuclease (Cat. No. TP790148) is the recombinant Cas9 protein purified from E.coli cell with a N terminal His tag and Simian virus 40 (SV40) nuclear localization sequence (NLS) on both N- and C- termini of the protein.

OriGene Cas9 protein can be used in many different applications. It can be used for pre-screening of highly efficient gRNA sequence through in-vitro cleavage assays. It can also form a stable ribonucleoprotein (RNP) complex with the guide RNA (gRNA) component to perform in-vivo genome editing after transfection to target cells. In addition to functional assays, the purified Cas9 protein has been used successfully for the production of specific monoclonal antibody (Cat. No. TA811179).

Package Contents and Storage Conditions

- Each tube contains 82.5ug purified recombinant protein of S. pyogenes Cas9 nuclease
- Ship on dry ice and store at -20°C upon arrival.

Key Features

- **High Protein Purity:** > 95% pure as determined by SDS-PAGE with Coomassie Blue detection.
- **Sterilized protein:** filtration sterilization and ready to be used for cell culture
- **Lack of non-specific Endonuclease Activity:** Cas9 protein is tested in a reaction with a supercoiled plasmid DNA. After incubation for 4 hours at 37°C, no significant nicking activity was observed by gel electrophoresis.
- **Lack of non-specific DNase Activity:** Cas9 protein is tested in a reaction containing a linearized DNA substrate. After incubation for 16 hours at 37°C, there is no significant degradation of the DNA substrate as determined by gel electrophoresis.
- **Lack of RNase Activity:** The protein is tested in a reaction with total RNA. After incubation for 1 h at 37°C, >95% of the substrate RNA remains intact as determined by gel electrophoresis.
- **High Endonuclease activity with specific sgRNA**: OriGene Cas9 Nuclease is tested in a reaction containing target DNA and synthetic sgRNA. 90% digestion of the substrate DNA is achieved after 1 hour incubation at 37°C.
- **Suitable for the in-vivo genome editing:** OriGene Cas9 Nuclease is co-transfected with specific sgRNA into RFP positive target cells. 10% cells have been successfully genetically modified to lose the RFP signal.

Other Features

Predicted MW	165.0 kDa
Concentration	20 uM
Buffer	10 mM Tris, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol pH 7.4 at 25°C
Endotoxin	< 0.5 EU per 1 µg of the protein by the LAL

Recommended materials

10X Cas9 Nuclease Reaction Buffer (100 ml, pH 6.5), Cat. No. GE100053

200mM HEPES solution

1M sodium chloride

50mM Magnesium chloride

1mM EDTA

Transfection reagent: Transfection reagents must be selected and optimized based on the cell type being used. For cells that are inherently difficult to transfect, direct electroporation of recombinant Cas9-sgRNA ribonucleoprotein (RNP) complexes is recommended. OriGene Cas9 protein (Cat. TP790148) can be used for standard electroporation methods.

Related Products

Cas9 Antibody

Type of antibody	Cat. No.	Species	Applications	Reactivities
Polyclonal	TA190309	Rabbit	WB, IP	S. Pyogenes
Monoclonal	TA811179	Mouse	WB, IP	S. Pyogenes
	TA811194	Mouse	WB, IP	S. Pyogenes
	TA811175	Mouse	WB,IP	S. Pyogenes
	TA811327	Mouse	WB, IP	S. Pyogenes
	TA160001	Mouse	WB, IF, IP	S. Pyogenes
HRP conjugated monoclonal	TA160002	Mouse	WB	S. Pyogenes
Carrier free (BSA/glyceol free) monoclonal	CF811194	Mouse	WB, IP	S. Pyogenes
	CF811179	Mouse	WB, IP	S. Pyogenes
	CF811327	Mouse	WB, IP	S. Pyogenes
	CF811175	Mouse	WB. IP	S. Pyogenes

Cas9 expression plasmid

Type of plasmid	Cat. No.	Name	Promoter
All-in-one Cas9/gRNA plasmid	GE100002	pCas-Guide	CMV
	GE100018	pCas-Guide-EF1a-GFP	CMV
	GE100022	pCas-Guide-EF1a-CD4	CMV
	GE100010	pLenti-Cas-Guide	CMV
	GE100045	pLenti-EF1a-Cas-Guide	EF1a
Cas9 only plasmid	GE100037	pAAVS1-Cas9-Puro-DNR	CMV
	GE100039	pAAVS1-Cas9-BSD-DNR	CMV
	GE100028	pLenti-Cas9	CMV
	GE100029	pLenti-Cas9-IRES-Puro	CMV
	GE100030	pLenti-EF1a-Cas9-IRES-Puro	EF1a
	GE100031	pLenti-Cas9-P2A-tGFP	CMV
	GE100014	pT7-Cas9	T7

Cas9 mutant

Type of product	Cat. No.	Name	Mutation
Protein	TP790151	CAS9-D10A S. pyogenes Recombinant Protein	D10A
Expression plasmid	GE100019	pCas-Guide-Nickase (D10A)	D10A
	GE100020	pT7-Cas9-Nickase (D10A)	D10A

Experimental Protocols

In vitro digestion of DNA with Cas9 nuclease and sgRNA

Overview:

This protocol describes how to digest double-stranded DNA *in vitro* using OriGene Cas9 protein (TP790148) and a synthetic guide RNA (sgRNA).

Required Materials:

- Cas9 Nuclease, S. pyogenes (Cat. No. TP790148)
- 10X Cas9 Nuclease Reaction Buffer (Cat. No. GE100053, 200mM HEPES, 1 M NaCl, 50mM MgCl2, 1mM EDTA, pH 6.5 at 25°C, pH is critical for Cas9 activity, make sure buffer has the right pH value).
- Nuclease-free water
- sgRNA containing the targeting sequence (GATGCAGCCGTTCTGGAAGC) against turboFRP gene
  - ✓ Synthetic pure sgRNA can be conveniently ordered from OriGene sgRNA service. http://www.origene.com/CRISPR-CAS9/
  - ✓ Alternatively, sgRNAs can be generated by *in vitro* transcription Kit using linearized plasmid, PCR products, or oligonucleotides as templates
  - ✓ sgRNAs must contain sequence complementary to the target DNA (guide sequence)
- DNA substrate containing turboRFP sequence (Cat. No. PS100074, pLenti-C-tRFP cloning vector)
  - ✓ Substrate can be circular or linearized plasmid, PCR products, or synthesized oligonucleotides
- Equipment and Reagents for DNA fragment analysis (agarose, DNA loading dye, Gel electrophoresis apparatus, etc.).

Before Starting

- We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination.
- Reconstitution of synthetic sgRNA (1nmol, target sequence: GATGCAGCCGTTCTGGAAGC)
  - ➢ Spin down briefly before opening the tube.
  - ➢ Add 100µl nuclease-free water to make 10µM stock solution.
  - ➢ Dilute stock solution to make 1µM working solution.

- Prepare 200-400 ng linearized plasmid DNA (Cat. No. PS100074) as substrate.

Procedure

1. Assemble the reaction in a nuclease free PCR tube in the following order:

Component	Volume (30ul final)
Nuclease-free water	21 µl
10X Cas9 Nuclease Reaction Buffer	3 µl
1 µM sgRNA	1 µl (~30 nM final)
1 µM Cas9 Nuclease, S. pyogenes (TP790148)*	1 µl (~30 nM final)
20 nM substrate DNA	4 µl (~3 nM final)
Total reaction volume	30 µl

* 1 µM equals 165 ng/µl.

2. Mix thoroughly and spin down briefly.
3. Incubate at 37°C for 1 hour, then heat at 65°C for 10 min to deactivate Cas9 nuclease. Hold the reactions at 4°C if you want to take a break.
4. Proceed with fragment analysis by gel electrophoresis.

Result

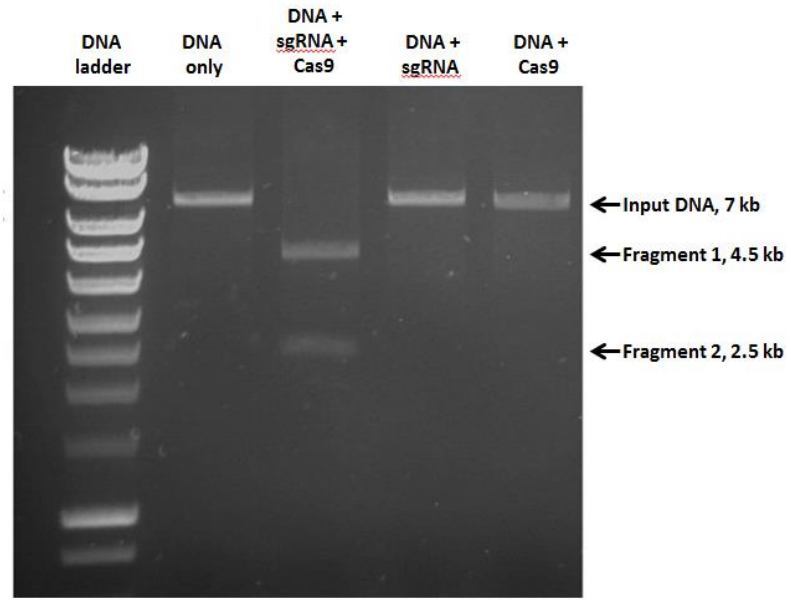

Figure 1: In-vitro digestion of PS100074 vector with purified Cas9 protein (Cat. No. TP790148) and specific sgRNA. Purified Cas9 protein can cut the substrate DNA only when it is incubated with specific sgRNA.

In vivo genome editing with Cas9 nuclease and sgRNA

Overview:

This protocol describes how to edit host cell genome *in vivo* using OriGene Cas9 protein (TP790148) and a synthetic guide RNA (sgRNA).

Required Materials:

- Cas9 Nuclease, S. pyogenes (Cat. No. TP790148, 20 µM)
- sgRNA containing the targeting sequence (GATGCAGCCGTTCTGGAAGC) turboRFP (tRFP) gene
- Genetically modified HEK293 cells with the genome insertion of tRFP expression cassette
- Nuclease-free 1X TE buffer (Tris-EDTA pH7.4)
- Transfection reagent: Lipofectamine® RNAiMAX Transfection Reagent (Thermo Fisher Cat No. 13778100)
- Opti-MEM Media® , Reduced Serum with Glutamax™ (Thermo Fisher Cat No. 51985091)
- Sterile 96-well tissue culture plates

Before Starting

- We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination.
- Dissolve sgRNA oligos in nuclease-free 1X TE buffer (Tris-EDTA pH7.4) to create a 10µM (10 pmol/ul) working stock.

Procedure

1. Form Cas9:sgRNA RNP complexes: For each transfection, mix 2µl (20 pmol) of sgRNA with 1µl (20 pmol) of Cas9 nuclease (Cat. No. TP790148, 20 µM). Add Opti-MEM Media to a final volume of 12.5 µl.
   Note that you may need to experimentally determine the optimum Cas9:sgRNA ratio for your cell type.
2. Incubate at room temperature for 15 minutes to assemble the RNP complexes.
3. To form each Transfection Complex, incubate the followings at room temperature for 30 minutes (perform step 4 during incubation).

Component	Volume (25 µl final)
RNP Complex (from step 2 above)	12.5 µl
Lipofectamine RNAiMAX Transfection Reagent	1.2 µl
Opti-MEM Media	11.3 µl
Total reaction volume	25 µl

4. During the 30-minute incubation, dilute cultured cells (tRFP positive genetically modified HEK293 cells) to approximately 4 X 10^5 cells/ml using complete media with no antibiotics.

5. Following incubation, add the 25 µl Transfection complex (from step 3) to each well of a sterile 96-well tissue culture plate

6. Add 125 µl diluted cells (from step 4) to each well of the 96-well tissue culture plate. Each well should contain approximately 5 X 10^4 cells and 10 nM of RNP complex.

7. Incubate the plate in the tissue culture incubator at 37°C (5% CO_2) for 48~72 hours

8. Determine the editing efficiency by observing the tRFP signal under microscope.

Result

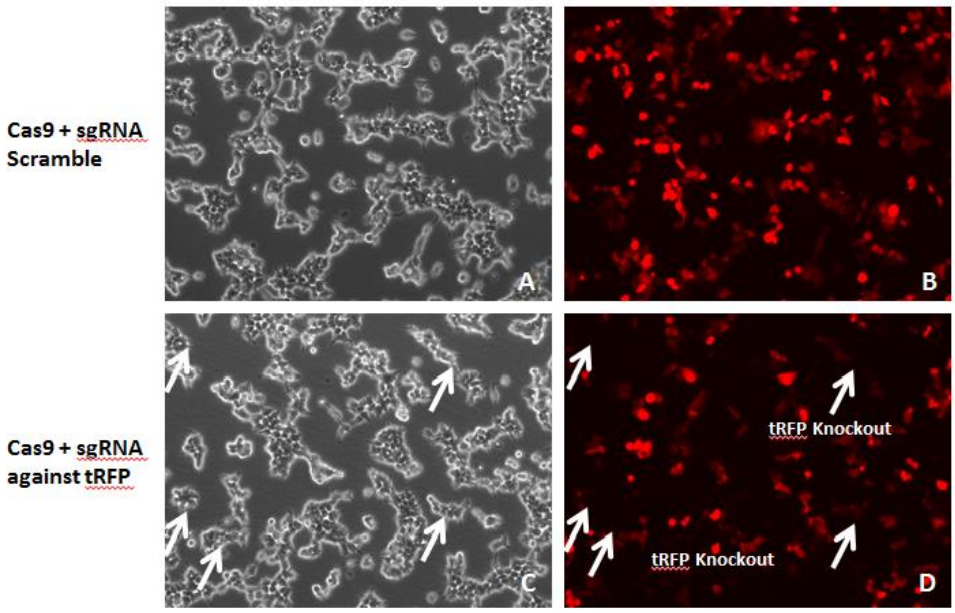

Figure 2: In-vivo genome editing of tRFP positive cells with purified Cas9 protein (Cat. No. TP790148) and specific sgRNA. Purified Cas9 protein can knockout the expression of tRFP protein in the tRFP postive cells when co-transefected with specific sgRNA.