Product Information

PURedit® Cas9 Protein

from Streptococcus pyogenes, recombinant, expressed in *E. coli*, 3X NLS

PECAS9

Product Description

PURedit® Cas9 recombinant protein was developed for the most efficient genome editing using RNP complex delivery. The protein is engineered with proprietary mutations that significantly improve editing activity while maintaining high on-target specificity. PURedit® Cas9 protein is manufactured using a verified process to guarantee high quality and reproducibility of every batch. The protein contains three varied nuclear localization sequences positioned for optimal activity. The molecular mass of PURedit® Cas9 is 162 kDa and the theoretical pI is 9.1.

Background Information

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system was discovered as a microbial adaptive immune system against invading viral and plasmid DNA. In this system, short DNA sequences (protospacers) from invading viruses are incorporated at CRISPR loci within the bacterial genome and serve as memory of previous infections. Reinfection triggers complementary mature CRISPR RNA (crRNA) to find a matching viral sequence.

Together, the crRNA and trans-activating crRNA (tracrRNA) guide CRISPR-associated (Cas) nuclease induce double-stranded breaks in the corresponding foreign DNA sequences.[1]

The type II prokaryotic CRISPR "immune system" has been engineered to function as an RNA-guided genome-editing tool that is simple, easy, and quick to implement.

Although the CRISPR system can be delivered to cells via plasmids, direct introduction of the Cas9:gRNA RNP complex strengthens and expands the applications of CRISPR genome modification technology by eliminating the possibility of plasmid DNA integration into the host genome. This method also results in fewer off-target effects due to the rapid degradation of the RNP after delivery; in many cases Cas9 RNP results in efficient genome modification with higher specificity when compared to cells transfected with Cas9 plasmid.[2-5] This RNP technology has broad applications and has been shown to work in both mammalian and plant systems.[6] Furthermore, Cas9 RNP delivery holds great promise for therapeutic applications including successful generation of knock-in primary human T cells.[7]

We offer a variety of lyophilized recombinant Cas9 proteins. All of these proteins can be combined either with SygRNA™ synthetic single guide RNA (sgRNA) or with synthetic crRNAs and tracrRNAs to form ribonucleoprotein (RNP) complexes that target the specific genomic locus of interest.

Components

Product	Quantity	Components	Catalogue number
PURedit® Cas9 Protein	1 vial	One vial contains lyophilized PURedit® Cas9 recombinant protein, 50 µg or 250 µg.	PEC9PR
PEXBUFF Transfection Enhancer for PURedit® Cas9 protein	1 vial	One vial contains 25 µL or 125 µL	PEXBUF
Reconstitution Solution 1 mL for Cas9 proteins	1 mL		RSOLUTION
Dilution Buffer for Cas9 proteins	1 mL		DBUFFER

Reagents and Equipment Required (Not provided)

- SygRNA™ synthetic single guide RNA (sgRNA) or synthetic crRNA and tracrRNA
- Electroporation System for Mammalian Cells

  Note: We recommend the Amaxa Nucleofector® 2b device (Lonza, AAB-1001) with Nucleofector® Kit (Lonza, different kits suitable for different cell lines)
- GenElute™ Mammalian Genomic DNA Miniprep Kit (G1N70)
- JumpStart™ *Taq* ReadyMix (P2893)
- Water, PCR Reagent (W1754)
- Custom DNA primers
- Mutation Detection
  - T7 Endonuclease Detection Assay (T7E1001)
  - NGS based analysis
  - Sanger based sequence analysis
- Gel Loading Buffer (G2526)
- Tris-Borate-EDTA Buffer, 5X concentrate, powdered blend (T3913)
- Ethidium Bromide Solution, 10 mg/mL in water (E1510)
- Appropriate cell culture media and cultureware

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The lyophilized protein is shipped on wet ice. Once resuspended in the provided Reconstitution Solution, the proteins are recommended to be stored at −20 °C or at −80 °C for long term storage.

Preparation Instructions

Minimum deliverable protein quantities are listed as package sizes. Precise quantities vary by lot number; please refer to the Certificate of Analysis for exact protein amounts per vial.

It is CRITICAL to resuspend the lyophilized proteins in the recommended volume of Reconstitution Solution to maintain the correct formulation for optimized stability. Do NOT use more than the recommended volume. Increasing the volume will reduce salt concentration that is essential for Cas9 protein stability. If lower protein concentrations are desired, the reconstituted proteins can be further diluted with the provided Dilution Buffer (DBUFFER).

The Reconstitution Solution provided is 50% glycerol. In case this is not suitable for your specific application, it is recommended to use the provided Dilution Buffer, which does not contain glycerol.

1. Resuspend the lyophilized protein with the supplied Reconstitution Solution (RSOLUTION).
   - For 250 µg vials, add 50 µL of Reconstitution Solution to achieve a concentration of ~5 mg/mL.
   - For 50 µg vials, add 30 µL of Reconstitution Solution to achieve a concentration of ~1.7 mg/mL.
   - Refer to Table 1 for the weight to picomole conversion of the PURedit® Cas9 protein.

Weight (µg)	Picomole (pg)
1.7	10.5
5	30.8

Table 1. Weight to picomole conversion of the PURedit® Cas9 protein.

2. Gently flick the tube to dissolve lyophilized powder and place the tube on ice for at least 30 minutes with flicking 2-3 times during incubation to ensure a homogeneous solution. Spin the tube to bring material to bottom of tube.
3. After reconstitution, protein should be stored at −20 °C for frequent uses. Alternatively, protein can be stored at −80 °C for long term storage. If the lyophilized protein is reconstituted in the Dilution Buffer (see Note above), it cannot be stored at –20 °C and freeze-thawed in the absence of glycerol.
4. If a lower concentration of Cas9 protein is required, dilute the Cas9 protein with supplied Dilution Buffer (DBUFFER) immediately before use. Store diluted protein on ice for up to 6 hours. Discard the diluted protein after use. Diluted protein cannot be stored at −20 °C.

Procedure

Researchers should use their preferred method to introduce Cas9 RNP into the cells of interest.

We provide a variety of transfection reagents, synthetic sgRNA, cell culture media and plates, and custom DNA primers for detection of CRISPR-mediated genome editing. For your reference, suggested protocols are described below.

Procedure Overview and Recommendations

In general, the steps required for successful introduction of Cas9 RNP into immortalized and primary cells are as follows:

1. Preparation of cells. Plate cells in complete growth medium approximately 18-48 hours before use, depending on cell types. For most cell types, cultures should be about 50-80% confluent at the time of transfection.
2. Preparation of guide RNA reagents.
   - The guide RNA can be synthetic or in vitro transcribed (IVT). We provide custom SygRNA™ synthetic single guide RNA (sgRNA) or synthetic crRNA and tracrRNA.
   - When using synthetic crRNA and tracrRNA, the two RNA molecules should be used in a molar ratio of 1:1. Annealing of the crRNA and tracrRNA is optional.

3. Assembly of Cas9 RNP complex.
   - Assemble guide RNA and Cas9 protein complex (RNP complex) on ice and incubate at room temperature for 10-15 minutes. If RNP samples are stored on ice after complexing, warm up the samples to room temperature for 5-10 minutes prior to transfection.
   - It is recommended to prepare RNP in a molar ratio between 1:1 to 5:1 (guide RNA: Cas9 protein). Further optimization of guide RNA: Cas9 protein ratio may be required.
4. Cell transfection with Cas9 RNP complex. Transfect the Cas9 RNP into the cells with the chosen transfection reagent.
5. Harvest transfected cells and perform mutation detection.
   - Allow the cells to grow 48-72 hours post-transfection before harvesting.
   - There are many methods to detect indels produced by CRISPR systems. The most used methods include mismatch detection assay using T7E1 Endonuclease Detection Assay (T7E1001), NGS based sequence analysis, and Sanger based sequence analysis.

RNP Preparation and Nucleofection (6-Well Plate Format)

1. Prepare SygRNA™ and PURedit® Cas9 protein RNP complex:
   - If using SygRNA™ single guide RNA, reconstitute the single guide RNA to 100 µM (100 picomole/µL) in 10 mM Tris buffer, pH 7.4.
   - If using SygRNA™ crRNA and tracrRNA, reconstitute SygRNA™ crRNA and tracrRNA each to 100 µM (100 picomole/µL) in 10 mM Tris buffer, pH 7.4.
   - Optional step: Anneal the crRNA and tracrRNA by incubating the mixture for 5 minutes at 95 °C, then placing the mixture on ice for 20 minutes.
   - Pipette 5 µL of the supplied Dilution Buffer to a sterile microcentrifuge tube on ice. The volume of Dilution Buffer may be adjusted according to the desired final RNP volume.
   - Add the desired amount of guide RNA to the tube. The RNA amount depends on the amount of Cas9 protein used and the desired guide RNA: Cas9 protein ratio.
   - Add the desired amount of PURedit® Cas9 protein (30-50 picomole) to the tube and mix gently by flicking. Spin the tube briefly and incubate at room temperature for 10-15 minutes. If RNP samples are stored on ice after complexing, warm up the samples to room temperature for 5-10 minutes prior to transfection. The final volume of RNP complex should not exceed 15 µL. If desired, dilute PURedit® Cas9 protein to a desired concentration using the supplied Dilution Buffer before RNP assembly. Store diluted PURedit® Cas9 protein on ice for up to 6 hours. Do not freeze diluted PURedit® Cas9 protein.
2. Prepare Nucleofector® Solution and cells:
   - Prepare Nucleofector® Kit reagents according to manufacturer's instructions. Bring nucleofection solution to room temperature before experiment.
   - Add 2 mL of complete medium to each well of a 6-well plate and prewarm the plate at 37 °C and 5% CO_2 until use.
   - Obtain enough cells for ~2.5 x 10^5 cells per nucleofection.
   - Collect the cells by centrifugation and remove the medium by aspiration.
   - Wash the cells twice with Hanks' Balanced Salt Solution.
   - Transfer an aliquot of Nucleofector® Solution (with Supplement added) needed for the experiment to a sterile tube. Add 0.5 µL or 1 µL of Transfection Buffer (PEXBUF) per 100 µL and mix thoroughly.

     Note: Optimal amount of PEXBUF buffer may depend on cell type used.
   - Resuspend the cells with the prepared Nucleofector® Solution at ~2.5 x 10^5 cells per 100 µL of solution.

     Note: Limit the exposure of cells to the Nucleofector® Solution to less than 30 minutes to ensure the best cell fitness. Plan the experimental steps accordingly. It is recommended to resuspend the cells in the Nucleofection Solution AFTER Cas9 RNP complex has been prepared.

3. Transfect cells with Cas9 RNP complex.
   - Pipette 100 µL of resuspended cells from Step 2 above to the tube containing Cas9 RNP complex and pipette up and down gently to mix completely. Avoid introducing air bubbles.
   - Transfer the mixture to a nucleofection cuvette.
   - Perform electroporation immediately using the appropriate Nucleofector® program according to manufacturer's recommendation.
4. Transfer nucleofected cells to medium.
   - Immediately add a pipette-full (~500 µL) of prewarmed medium from a well of the 6-well plate to the cuvette and gently transfer the sample back to the same well. Use the pipettes supplied with the Nucleofector® kit and avoid repeated aspiration of the sample.
   - Allow cells to grow for 48-72 hours at 37 °C and 5% CO_2 before harvesting for assay. It is not necessary to replace the medium.

Preparation and Microinjection of Cas9/SygRNA™ RNP into One-Cell Embryo

Microinjection protocols vary greatly depending on embryo type and researcher preferences. Microinjection of Cas9 RNPs has been demonstrated in the following organisms:

- *Caenorhabditis elegans* (nematode)[8]
- *Mus musculus* (mouse)[9,10]
- *Rattus norvegicus* (rat)[10]
- *Danio rerio* (zebrafish)[11]

Related Cas9 Protein Products

- Cas9 Plus Protein, from *Streptococcus pyogenes*, recombinant, expressed in *E. coli*, 3X NLS (CASPL).
- Wild type *Streptococcus pyogenes* Cas9 Protein (CAS9PROT).
- Cas9-GFP Protein from *Streptococcus pyogenes*, fused with enhanced GFP, recombinant, expressed in *E. coli*, 3X NLS (CAS9GFPPRO).

References

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11. Kotani, H., et al., Efficient multiple genome modifications induced by the crRNAs, tracrRNA and Cas9 protein complex in zebrafish. PLoS ONE, 10 (2015).
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Troubleshooting Guide

Suspected Issue	Solution
The PURedit® Cas9 protein has denatured after long term storage in Dilution Buffer.	The provided Dilution Buffer is recommended only for immediate use. For long term storage, keep the protein lyophilized or resuspended in the provided Reconstitution Solution at −20 °C.
The PURedit® Cas9 protein has been thawed and refrozen too many times.	The protein is sensitive to several rounds of temperature cycling. Aliquoting and/or stable low temperature storage methods will allow for this potential issue to be avoided.
The crRNAs and tracrRNAs need to be annealed before complexing with the PURedit® Cas9 protein.	While an annealing step is generally not needed, it has been shown to increase cutting in rare cases.[12] To anneal the crRNA and tracrRNA, mix them in the desired ratio and incubate the mixture for 5 minutes at 95 °C, then place the mixture on ice for 20 minutes.
The crRNAs and tracrRNAs are degraded.	Under normal cell culture conditions, synthetic RNA modifications are not needed; however, for certain cell lines, this may be necessary. Modifications are available through us.
The transfection or nucleofection is not working or is too toxic.	For any transfection reagent or nucleofection, the protocol should be optimized for each cell line used. Refer to the manufacturer's protocol for further assistance.
IVT RNA is low quality or degraded.	For optimal performance, only quality verified IVT RNA should be used. For mammalian cell transfection, remove the 5' triphosphate group with a phosphatase to avoid cell toxicity.

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