Product Information

PURedit® Cas9 Cytosine Base Editor Protein Kit for RNP-Based Precision Genome Editing

Recombinant, Expressed in *E. coli*, 3X NLS

Flexible Editor-CAS9CBEFLX

Precision Editor-CAS9CBEPRC

Product Description

Our Flexible and Precision cytosine base editor proteins were developed to enable cytosine base editing at high rates while minimizing the rate of off-target and genome-wide effects by using RNP complex delivery. The proteins were engineered with complementary editing windows: the Flexible base editor enables flexibility in positioning the target cytosine within the target site, while the Precision base editor minimizes the rate of editing of nearby (non-target) cytosine residues. Both base editors utilize a nickase Cas9 domain from *Streptococcus pyogenes* to maximize the rate of editing while minimizing the rate of double strand breaks. Cytosine base editor proteins are manufactured using a verified process to guarantee high quality and reproducibility of every batch. Uracil glycosylase inhibitor (UGI) protein is included as a recommended optional component to enhance the rate of cytosine-to-thymine editing and reduce the rate of other editing outcomes, including insertions and deletions (indels).

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.[1] 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 dsDNA sequence and cleave it, inactivating the virus.

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. Cleavage of a genomic target by a CRISPR effector such as Cas9 can result in the installation of indels, leading to frameshift mutations. If the cell is provided with a DNA repair template, homology-dependent repair (HDR) can install a precision edit, including specific single-nucleotide substitutions. However, many challenges exist with HDR: 1) HDR activity is cell cycle-dependent 2) HDR rates are low in many cell types 3) a dsDNA repair template has a small chance of nonspecific or off-target insertion into the cell genome and 4) dsDNA repair templates are toxic in some cell types, such as T cells.

To circumvent these challenges, base editing was developed to install precision substitutions without double-strand breaks at rates superior to HDR.[2,3] A cytosine base editor consists, minimally, of a dead or nickase CRISPR effector domain (such as Cas9) and a cytosine deaminase domain. A target-specific guide RNA directs the Cas9 domain to bind to the target dsDNA, where the cytosine deaminase converts cytosine residue(s) to uracil. When mismatch repair or DNA replication uses this uracil as a template, it results in the installation of an adenine on the opposite strand. The result is a cytosine-to-thymine substitution.

Cells utilize base excision repair (BER) to correct damage events that result in the presence of uracil in DNA. In the context of cytosine base editing, this has several adverse effects: 1) reversion of a successful edit to the unedited sequence 2) cytosine-to-purine substitutions and 3) the formation of indels. Uracil glycosylase inhibitor (UGI) blocks the first step of BER, maximizing the rate of C-to-T substitution and reducing the rate of C-to-R substitution and indel. We include purified UGI protein as an optional component to enable the experimenter to choose whether to maximize C-to-T substitution or allow C-to-R substitution, as appropriate for their experimental needs.

Although the base editor system can be delivered to cells via plasmids, direct introduction of the base editor: 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 base editor RNP results in efficient genome modification with higher specificity when compared to cells transfected with base editor plasmids.[4,5,6]

Base Editor Product Details

Our Flexible and Precision cytosine base editors were engineered with complementary editing windows to suit our customers' different experimental needs.

The Flexible Editor will edit cytosine residues from the third protospacer position to the 15th position (sixth position from the PAM). The Precision Editor will edit cytosine residues from the third protospacer position to the ninth position. If experimental needs require flexibility in placement of the target cytosine within the target sequence, it is recommended to use the Flexible Editor. If a target sequence contains additional cytosine residues that should not be edited, it is recommended to use the Precision Editor, positioning the target cytosine within the editing window and non-target cytosine(s) outside the editing window (Figure 1).

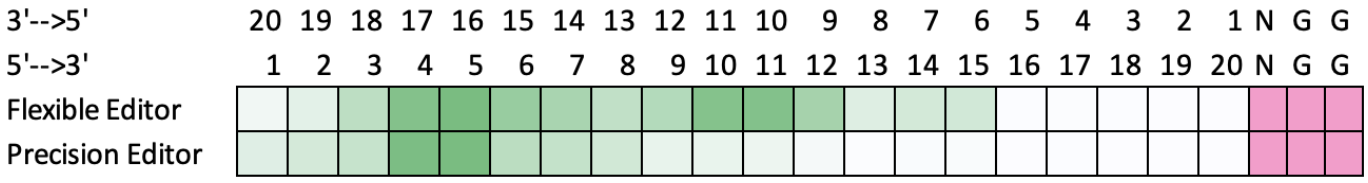

Figure 1: Editing windows for Flexible and Precision editors. Darker shades of green indicate higher editing activity.

The deaminase in our cytosine base editor proteins exhibits sequence context preferences that affect editing efficiency. Cytosine residues preceded by thymine residues (TC dinucleotides) are edited most strongly, followed by CC dinucleotides, followed by AC and GC dinucleotides (Figure 2). This characteristic should be factored into guide design. Care should be taken to locate any non-target cytosine residues in a TC context outside the editing window to prevent unwanted editing at these residues, particularly if the target cytosine is not in a TC context. In contrast, non-target cytosine residues in an AC or GC context may not need such stringent placement outside the editing window, particularly if the target cytosine is in a TC context.

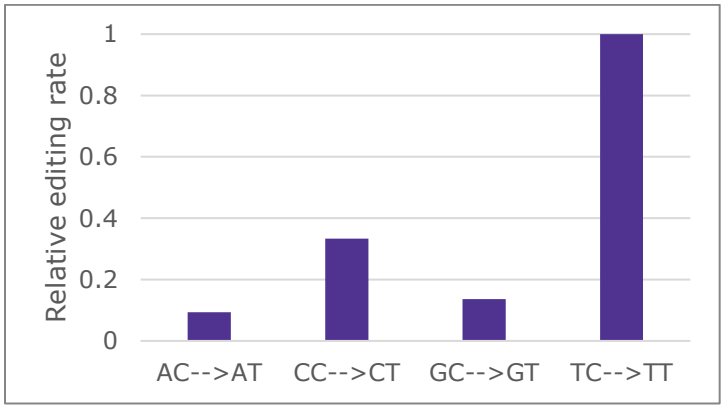

Figure 2: Dinucleotide sequence editing preference. Cytosine residues preceded by thymine (TC dinucleotide) are edited more strongly than residues preceded by cytosine (CC), adenine (AC), or guanine (GC).

We have developed a guide design tool to facilitate experimental planning and editor selection. This tool uses the editing windows and sequence context preferences discussed above to computationally predict the relative rates of editing at cytosine residues within a user-defined sequence.
The tool can be found at: SigmaAldrich.com/BaseEditorDesign.

We include UGI as an optional component to enable flexibility in editing outcomes. For traditional C-to-T editing, inclusion of UGI is strongly recommended to reduce the rates of C-to-R substitution and indel. If C-to-R substitutions are desired, the user may elect to omit UGI from the transfection to enable the formation of these outcomes.

We also provide PEXBUFF, a non-nucleic acid polymer transfection enhancer, which can boost transfection efficiencies without risk for DNA integration into the target cell genome. In base editing, PEXBUFF increases the rate of all editing outcomes and can be combined with UGI protein for optimal results.

	Flexible Editor	Precision Editor
Molecular Weight	189 kDa	214 kDa
Picomoles per microgram protein	5.29 pmol/µg	4.67 pmol/ µg

Table 1: Weight to picomole conversion for base editor proteins

Components

Components Provided	Quantity	Catalog number	Comments
Cytosine base editor protein	1 vial	Flexible-CAS9CBEF Precision-CAS9CBEP	One vial contains cytosine base editor recombinant protein in 50% glycerol at 8 mg/mL, 30 reactions or 90 reactions.
Uracil glycosylase inhibitor (UGI) protein	1 vial	UGIPRT	One vial contains UGI recombinant protein in 50% glycerol at 15 mg/mL, 30 reactions or 90 reactions.
Transfection Buffer	1 vial	PEXBUF	One vial contains 25 µL or 125 µL.
Dilution Buffer for Cas9 proteins	1 vial	DBUFFER	-

Materials Required (Not provided)

- SygRNA™ synthetic single guide RNA (sgRNA) or synthetic crRNA and tracrRNA
- Electroporation system for mammalian cells

  Note: We recommend the Lonza Nucleofector® 2b or 4D system (Lonza)
- 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 sequencing-based 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

Storage/Stability

The base editor and UGI proteins are recommended to be stored in their undiluted state at −20 °C for up to several months or at −80 °C for long-term storage. Avoid repeated freeze-thaw cycles. Proteins may be aliquoted for storage.

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.

Procedure

Researchers should use their preferred method to introduce Cas9 RNP into the cells of interest. Optimal amounts of UGI or PEXBUFF and ratios of guide RNA to base editor protein should be determined empirically.

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.

Pilot Study: As cell lines vary in their sensitivity, we recommend a pilot experiment with PEXBUFF transfection enhancer to determine optimal dosage for maximal cell viability.

Procedure Overview and Recommendations

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.
    a. The guide RNA can be synthetic or *in vitro* transcribed (IVT). Sigma-Aldrich provides custom SygRNA™ synthetic single guide RNA (sgRNA) or synthetic crRNA and tracrRNA.
    Order here: SigmaAldrich.com/customgrna.
    b. 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 but recommended.
3. Assembly of base editor RNP complex.
    a. It is recommended to use 30-50 pmol base editor protein (1 µL) per transfection. Increasing the input of base editor protein may increase editing rates; however, the higher glycerol content in RNP preparations with increased protein volume may reduce transfection efficiency.
    b. Gently mix together base editor protein, guide RNA, and, optionally, UGI protein. Do not vortex. Incubate at room temperature for 5-10 minutes. Store on ice until transfection.
    c. If RNP samples are stored on ice after complexing, warm the samples to room temperature for 5 minutes prior to transfection.
    d. It is recommended to prepare RNP in a molar ratio between 1:1 to 5:1 (guide RNA:base editor protein). Further optimization of guide RNA:base editor protein ratio may be required.
    e. Inclusion of UGI protein promotes the formation of C-to-T edits while minimizing the rate of indel and C-to-R edits. The optimal amount of UGI may need to be optimized on a site-to-site basis. It is recommended to begin with 15 µg (1 µL) of UGI protein per transfection. UGI can be diluted with the provided dilution buffer before use, if needed.
4. Cell transfection with base editor RNP complex.
    a. Transfect the base editor RNP into the cells with the chosen transfection reagent according to the manufacturer's instructions. Use of PEXBUFF transfection enhancer is recommended (detailed instructions can be found below).
5. Harvest transfected cells and perform mutation detection.
    a. Allow the cells to grow 48-72 hours post-transfection before harvesting.
    b. There are many methods to detect edits 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 guide RNA and base editor protein RNP complex.
    a. If using SygRNA™ single guide RNA (recommended), reconstitute the single guide RNA to 100 µM (100 picomole/µL) in 10 mM Tris buffer, pH 7.4.
    b. 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. Anneal crRNA and tracrRNA at a 1:1 ratio.
    c. 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. The final volume of RNP complex should not exceed 15 µL.
    d. Add the desired amount of guide RNA to the tube. The RNA amount depends on the amount of base editor protein used and the desired guide RNA: base editor protein ratio.
    e. Add the desired amount of base editor protein (recommended: 30-50 picomole, 1 µL) to the tube and mix gently by flicking. If necessary or desired, dilute base editor protein using the supplied Dilution Buffer before RNP assembly. Store diluted base editor protein on ice for up to 6 hours. Do not freeze diluted base editor protein.

f. Add the desired amount of UGI protein to the tube and mix gently by flicking. 15 µg (1 µL) typically yields increases in the rate of C-to-T edits and decreases in the rate of indels, but this may be titrated on a target-to-target basis for optimal performance.

Note: If C-to-R editing is desired, omit UGI.

g. Spin the tube briefly and incubate at room temperature for 5-10 minutes. If RNP samples are stored on ice after complexing, warm up the samples to room temperature for 5 minutes prior to transfection.

2. Prepare Nucleofector® Solution and cells.
   a. Prepare Nucleofector® Kit reagents according to manufacturer's instructions. Bring nucleofection solution to room temperature before experiment.
   b. Add 2 mL of complete medium to each well of a 6-well plate and pre-warm the plate at 37 °C and 5% CO_2 until use.
   c. Obtain enough cells for ~2.5 x 10^5 cells per Nucleofection or the cell amount recommended by the transfection reagent manufacturer's instructions.
   d. Collect cells by centrifugation and remove culture medium by aspiration.
   e. Wash the cells twice with Hank's Balanced Salt Solution.
   f. Transfer the volume of Nucleofector® Solution (with Supplement added) needed for the experiment to a sterile tube. Add 0.5 µL to 1 µL of PEXBUFF Transfection Buffer (PEXBUF) per 100 µL and mix thoroughly.

      Note: The optimal amount of PEXBUFF depends on the cell type and may require optimization for best results.

   g. Resuspend the cells with the prepared Nucleofector® Solution + PEXBUFF Transfection Buffer 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 20 minutes to ensure the optimal cell viability. To achieve this, it is recommended to prepare the base editor RNP complex before the cell suspension.

3. Transfect cells with base editor RNP complex.
   a. Pipette 100 µL of resuspended cells from step 2(g) above to the tube containing RNP complex and pipette up and down gently to mix completely. Avoid introducing air bubbles.

      Note: Many cell types settle quickly. Ensure cells are evenly distributed through the suspension before pipetting each transfection.

   b. Transfer the mixture to a Nucleofection cuvette.
   c. Perform electroporation immediately using the appropriate Nucleofector® program according to the manufacturer's recommendation.
4. Transfer nucleofected cells to medium.
   a. Immediately add a pipette-full (~500 µL) of pre-warmed 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.
   b. 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 culture medium.

Preparation of Microinjection of Base Editor 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 (not an exhaustive list):

- *Caenorhabditis elegans* (nematode)
- *Drosophila melanogaster* (fruit fly)
- *Mus musculus* (mouse)
- *Rattus norvegicus* (rat)
- *Danio rerio* (zebrafish)

Disposal and Recycling

Where services are available, cardboard packaging and cleaned polypropylene vials may be recycled.

Related Cas9 Protein Products

- Wild type *Streptococcus pyogenes* Cas9 Protein (CAS9PROT)
- Cas9 Plus Protein, from *Streptococcus pyogenes*, recombinant, expressed in *E. coli*, 3x NLS (CASPL)
- PURedit® Cas9 Protein, from *Streptococcus pyogenes*, recombinant, expressed in *E. coli*, 3x NLS (PECAS9)
- Cas9-GFP Protein from *Streptococcus pyogenes*, fused with enhanced GFP, recombinant, expressed in *E. coli*, 3x NLS (CAS9GFPPRO).

Troubleshooting Guide

If no editing is observed and there is reason to suspect an experimental flaw is at fault, the following considerations may aid the researcher in troubleshooting the experiment.

Problem	Suspected Cause	Solution
The wrong DNA strand was edited	Base editor protein edits the same strand of target DNA as contains the NGG PAM. The guide should be complementary to the unedited strand; in other words, the guide sequence should match the target strand sequence.	Re-design the guide RNA to target the correct strand. For assistance, use our base editor guide design tool on our website.
Nearby cytosine residues are edited in addition to (or in place of) the desired cytosine residue.	Non-target cytosine residues are located within the editing window.	If possible, re-design the guide RNA to place non-target cytosine residues outside the editing window. For assistance, use our base editor guide design tool on the website. If using the Flexible base editor, use the Precision base editor protein to enable editing with more precision.

Low editing rate	The target residue is not ideally placed in the editing window of the base editor protein.	Re-design the guide RNA to place the target residue more ideally in the editing window. For assistance, use our base editor guide design tool on the website. If using the Precision base editor, use the Flexible base editor protein to enable editing over a wider region of the target site.
	Base editor protein has degraded.	Proteins are sensitive to several rounds of temperature cycling. Aliquoting and/or stable low-temperature storage methods are recommended to prevent degradation. The provided Dilution Buffer is recommended only for immediate use. Diluted protein should not be frozen for later use.
	Guide RNA is degraded.	Under normal cell culture conditions, synthetic RNA modifications are not needed; however, for certain cell lines, this may be necessary. Modified sgRNAs are available through SigmaAldrich.com/customgrna. If using IVT RNA, only quality verified IVT RNA should be used. For mammalian cell transfection, remove the 5' triphosphate group with a phosphatase to avoid cell toxicity. Ensure reagents and equipment are certified RNase-free and take care not to introduce RNases. Clean surfaces and micropipettes with RNaseZAP™ if RNase contamination is suspected.
	Guide RNA is improperly complexed.	If using a 2-part crRNA and tracrRNA guide, anneal the crRNA and tracrRNA before complexing with base editor protein: 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. Ensure base editor protein and guide are incubated for 5-10 minutes at room temperature before transfection to facilitate proper RNP formation.

	Insufficient PEXBUF in transfections.	Perform PEXBUF titration to determine the optimal volume per transfection.
	Transfection protocol is not suitable.	Optimize transfection protocol for your cell line. Refer to the manufacturer's protocol.
No or insufficient improvement in the proportion of edits that are C-to-T or in indel rate	Insufficient UGI protein in RNP preparations	Perform UGI titration to determine the optimal volume per transfection.
No or insufficient C-to-R editing observed (when desired).	DNA repair processes at this target do not favor C-to-R substitution.	Omit UGI protein from RNP preparations. UGI inhibits the DNA repair processes responsible for C-to-R substitutions.
	UGI protein included in RNP preparations.	Ensure inclusion of PEXBUF in transfections to boost overall editing rate.
Toxicity is observed.	Too much PEXBUF in transfections.	Reduced the volume of PEXBUF or increase cell numbers per transfection. Limit the exposure of cells to transfection solution with PEXBUF, as prolonged incubation would result in lowered cells viability.
	Transfection protocol is not suitable.	Optimize transfection protocol for your cell line. Refer to the manufacturer's protocol.
	Cells exposed to transfection reagent for too long.	Some transfection reagents may be toxic to cells with long exposure. Work efficiently and/or perform transfections in batches to minimize the time cells are exposed to reagent.

References

1. M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna and E. Charpentier, "A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity," Science, vol. 337, no. August, pp. 816-822, 2012.
2. A. C. Komor, Y. B. Kim, M. S. Packer, J. A. Zuris and D. R. Liu, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage," Nature, pp. 420-424, 2016.
3. A. C. Komor, K. T. Zhao, M. S. Packer, N. M. Gaudelli, A. L. Waterbury, L. W. Koblan, Y. B. Kim, A. H. Badran and D. R. Liu, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity," Science Advances, vol. 3, no. 8, pp. 1-10, 2017.
4. H. A. Rees, L. C. Komor, W.-H. Yeh, J. Caetano-Lopes, M. Warman, A. S. Edge and D. R. Liu, "Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery," Nature Communications, vol. 8, p. 15790, 2017.
5. J. A. Zuris, D. B. Thompson, Y. Shu, J. P. Guilinger, J. L. Bessen, J. H. Hu, M. L. Maeder, J. K. Joung, Z.-Y. Chen and D. R. Liu, "Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo," Nature Biotechnology, vol. 33, no. 1, pp. 73-80, 2015.
6. S. Kim, D. Kim, S. W. Cho, J. Kim and J.-S. Kim, "Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins," Genome Research, vol. 24, pp. 1012-1019, 2014.

Limited Use Label License

Information on terms and conditions can be found at CRISPR Use License Agreement.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

23235425 Rev 07/25