User Guide

PEXBUFF Transfection Enhancer

PEXBUF

Product Description

PEXBUFF Transfection Enhancer (PEXBUFF) increases knockout efficiency or base editing of Cas9 ribonucleoprotein (RNP) complexes delivered by electroporation. It has been verified for use with Nucleofector® II/2b and 4-D System. Unlike other commercialized enhancers, PEXBUFF is a non-nucleic acid polymer and therefore presents no risk of DNA integration into the target genome. The effectiveness of PEXBUFF has been verified in several cell lines with multiple Cas9 proteins, including wildtype and high-fidelity variants. We recommend using PURedit® with Cas9/Cas9 Plus Protein to achieve the optimal editing efficiency while maintaining high on-target specificity.

Component

25 µL or 125 µL of PEXBUFF Transfection Enhancer

Storage/Stability

Store the buffer at -20 °C.

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.

Reagents and Equipment Required

(not provided)

Items listed below can be purchased at SigmaAldrich.com unless otherwise stated.

- SygRNA® synthetic single guide RNA (various catalog numbers) or synthetic crRNA (Synthetic crRNA) and tracrRNA (TRACRRNA05N)
- Cas9 Plus Protein (CAS9PL) or PURedit® Cas9 Protein (PECAS9)
- Electroporation System for Mammalian Cells

  Note: We recommend the 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* Ready Mix (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, 5× concentrate, powdered blend (T3913)
- Ethidium Bromide Solution, 10 mg/mL in water (E1510)
- Appropriate cell culture media and cultureware

Procedure

We recommend using Nucleofector® II/2b or 4-D System for the transfection.

Follow the steps (below) for successful administration of PEXBUFF Transfection Enhancer in **6-well format**.

Preparation of cells

Split cells in complete growth medium approximately 24–48 hours before use, depending on the cell types. For most cell types, cultures should be about 50–80% confluent at the time of transfection. If starting from frozen culture, it's recommended to grow the culture for at least one week to allow ample revival time.

Preparation of Medium Plates for Transfection

1. Prepare the medium plates by adding 2 mL of complete growth medium into each well.
2. Prewarm the plates at 37 °C and 5% CO_2 until use.

Preparation of Guide RNA Reagents

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.

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.

Assembly of Cas9 RNP complex

1. 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.
2. 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: Anneal the crRNA and tracrRNA by incubating the mixture for 5 minutes at 95 °C, then placing the mixture on ice for 20 minutes.
3. Pipette 5 µL of the Dilution Buffer supplied with the protein to a sterile 500 µL microcentrifuge tube on ice. The volume of Dilution Buffer may be adjusted according to the desired final RNP volume.
4. 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. Prepare RNP in a molar ratio between 3:1 to 5:1 (guide RNA:Cas9 Protein). Further optimization of guide RNA:Cas9 Protein ratio may be required.
5. Add the desired amount of PURedit® Cas9 Protein (30-50 picomole) to the guide RNA 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/Cas9 Plus Protein to a desired concentration using the supplied Dilution Buffer before RNP assembly. Store diluted PURedit® Cas9/Cas9 Plus Protein on ice for up to 6 hours. Do not freeze diluted PURedit® Cas9 or Cas9 Plus Protein.

Cell Transfection with Cas9 RNP Complex

1. Wash cells with Hank's Balanced Salt Solution and add trypsin solution after the wash to detach the cells. For suspension cultures, skip this step and go to Step 2.
2. Measure the cell concentration and transfer the desired number of cells for each transfection experiment to a 50 mL conical tube and spin down the cells at 1,000 rpm for 5 minutes at room temperature.
3. Wash the cells twice with Hank's Balanced Salt Solution.
4. Prepare a single 500 µL microcentrifuge tube for each transfection sample. Add 0.5 µL of PEXBUFF for attached cells and 1 ul for suspension cells. (The amount of PEXBUFF can be optimized for each cell line.)
5. Resuspend the cells with 100 µL transfection buffer or the desired amount per transfection protocol.
   Note: If less volume of transfection buffer is used, reduce the volume of PEXBUFF proportionally. PEXBUFF can be diluted with sterile water for ease of pipetting.
6. Carefully mix the cells in transfection buffer with the PEXBUFF, pipetting at least six times. Transfer the cells containing PEXBUFF to the tube with RNP complex and mix carefully and thoroughly.
7. Perform transfection.
8. Return transfected cells to incubator as soon as the plate is completed. Allow cells to grow for 48–72 hours at 37 °C and 5% CO_2 before conducting editing analysis. It is not necessary to replace the medium within this period.
9. Analyze editing results by T7E1 Endonuclease Detection Assay, Sanger based sequence analysis or NGS.

Note:

- For best results, it is recommended to add PEXBUFF to the cells before each transfection. Making a master mix of the cells and PEXBUFF or prolonged incubation of cells in PEXBUFF containing transfection buffer might diminish the editing enhancement generated by the PEXBUFF.
- Each cell line has different sensitivities to PEXBUFF. Optimization of cell numbers per transfection and titration of the PEXBUFF to determine the optimal amount to use based on the target sequence and cell type is recommended.
- For the strip tube format or 96-well transfection format of the Nucleofector® 4-D system, the volume of PEXBUFF should be scaled according to the volume of corresponding transfection solution.
- As mammalian cells settle quickly, always make sure the cells are well suspended before performing transfection of each sample.

Troubleshooting Guide

Suspected Issues	Solution
Toxicity is observed.	Reduce the volume of PEXBUFF or increase cell numbers per transfection. Higher toxicity and slower cell growth might be observed when the editing level is high. Limit the exposure of cells to transfection solution with PEXBUFF, as prolonged incubation may result in lowered cells viability.
No improvement of editing efficiency.	Titrate PEXBUFF to determine the optimal volume per transfection and sequence.

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