supra-ABE

Catalog #CAS-EE145

Product Information

Product name: supra-ABE

Catalog #: CAS-EE145

Express System: *E. coli*

Product Grade: RUO

Concentration 10 mg/mL

Purity(SEC-HPLC) ≥ 80%

Molecular Weight:188.1kDa

Endotoxin: ≤ 10.0 EU/mg

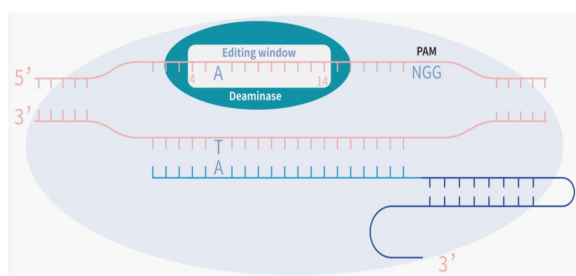
Size: 100μl/1mg

Form: Liquid

Storage Buffer: 30mM Tris, 300mM NaCl, 50% Glycerol, 0.1mM EDTA, 5mM DTT, pH 7.8

Storage/Transport Transport on dry ice. Store at -80 ±10°C. Avoid repeated freezing and thawing

Product Description: supra-ABE is an adenine base editor comprising the exclusively patented adenine deaminase eMa-TadA fused with Cas9 nickase (Cas9n), where eMa-TadA is embedded within nCas9. The eMa-TadA deaminase catalyzes the deamination of adenine in DNA, converting it to inosine. During DNA replication, inosine is recognized as guanine, enabling A-to-G base substitution within editing window positions 4-14 without requiring a donor template or inducing DNA double-strand breaks. The supra-ABE system demonstrates high editing activity, broad effective editing windows, and low off-target rates, making it a versatile tool for applications in cell and gene therapy.



Editing window of supra-ABE

Applications

1. Gene Knockout

Gene knockout is achieved by mutating the start codon ATG or altering the canonical splice sites GT-AG through A-to-G or T-to-C substitutions, thereby disrupting gene function.

2. Gene Correction

Precise repair of pathogenic single-base mutations (A-to-G or T-to-C substitutions at specific adenine or thymine sites) enables restoration of normal gene function through single-base editing.

Quality Specifications

Item	Acceptance Criteria
Concentration	9.0-11.0mg/mL
Purity (Bis-Tris PAGE)	≥ 80.0%
Purity (SEC-HPLC)	≥ 80.0%
Endotoxin	≤ 10.0EU/mg

Performance Data

Purity (Bis-Tris PAGE)

Fig. 1 Bis-Tris PAGE analysis confirms supra-ABE purity exceeding 80%

Purity (SEC-HPLC)

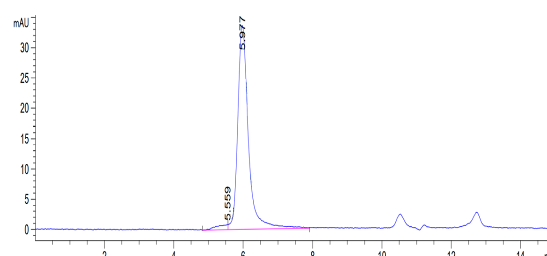


Fig.2 SEC-HPLC analysis confirms supra-ABE purity exceeding 80%.

电话：400-614-0008
网站：www.kactusbio.cn

Base editing efficiency

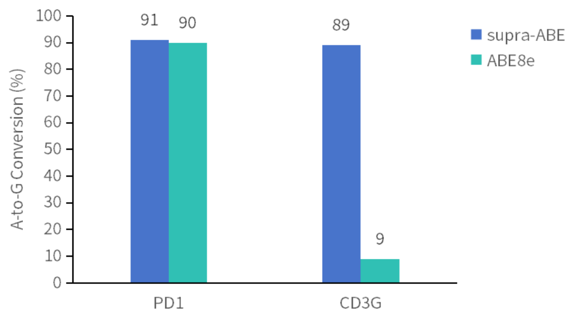


Fig.3 Sanger sequencing and EditR analysis demonstrate that supra-ABE efficiently mediates A-to-G conversion in the start codon (ATG) of PD1 and CD3G genes, disrupting translation initiation and achieving approximately 90% knockout efficiency.

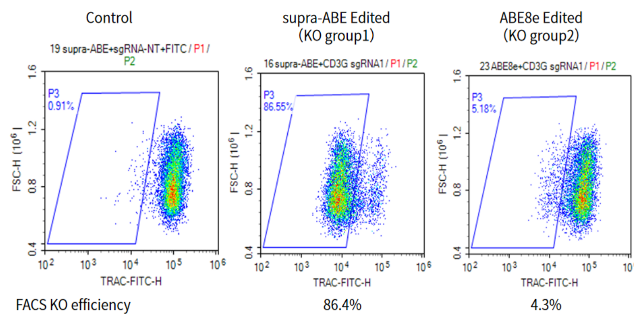


Fig.4 FACS analysis demonstrates that supra-ABE efficiently knocks out TRAC protein with 86.4% knockout efficiency, significantly outperforming ABE8e

Protocol

I. Protocol for T Cell Gene Knockout

1. T Cell Activation

1.1 Thaw T cells following standard protocols, resuspend in 10 mL medium, and centrifuge at 300 × g for 5 min at room temperature (RT).

1.2 During centrifugation, prepare Dynabeads™ Human T-Activator CD3/CD28: Vortex beads to resuspend, transfer 50 μL beads (1:1 ratio for 2E+06 cells) to a sterile flow tube. Add 2 mL PBS, vortex for 5 s, place tube on a magnet for 1 min, and discard supernatant. Resuspend washed beads in 2 mL medium.

1.3 Mix cells with resuspended Dynabeads and transfer to a 6-well plate for culture.

1.4 Monitor cell growth and maintain cell density at 0.8E+06 cells/mL.

1.5 On day 4 post-activation, resuspend cells in flow tubes, isolate beads using a magnet, transfer cells to 15 mL centrifuge tubes, count, centrifuge, and prepare for electroporation.

2. Cell Electroporation

2.1 Reconstitute sgRNA dry powder with nuclease-free water to 2–4 μg/μL and store.

2.2 Prepare RNP complex: In a 1.5 mL EP tube, mix 5.0 μg supra-ABE protein with 2.0 μg sgRNA, incubate at 37°C for 15 min.

2.3 Add 20 μL electroporation buffer (3.6 μL Solution 1 + 16.4 μL Solution 2) containing 0.8E+05 cells to the RNP mixture. Gently mix with a pipette to avoid bubbles.

2.4 Transfer the entire mixture (≤26 μL) to a cuvette without bubbles.

2.5 Perform electroporation using the Lonza 4D-Nucleofector™: Select the X module, load cuvette, choose "T cell, human, stimulated" settings, and run program EO115. Remove cuvette and shut down the instrument post-electroporation.

2.6 Add 120 μL 1× dual-antibiotic complete medium to the cuvette, incubate for 15 min, transfer cells to a 6-well plate, rinse cuvette with 120 μL medium, and add to the plate. Adjust total medium volume to 3 mL and culture.

3. Flow Cytometry Analysis

3.1 After 72 h, observe cell morphology under a microscope and harvest cells for genomic extraction and flow cytometry.

3.2 Collect 5E+05 cells per group into 1.5 mL EP tubes, centrifuge at 300 × g for 5 min (RT), and discard supernatant. Wash once with PBS.

3.3 Prepare antibody staining: Dilute antibody 1:50 in PBS containing 2% BSA (1 μL stock + 50 μL dilution buffer). Add to tubes and incubate at 4°C in the dark for 20 min.

3.4 Wash twice with PBS and analyze editing efficiency by flow cytometry.

4. Genomic Extraction and Sequencing Analysis

4.1 Extract genomic DNA from harvested cells and PCR-amplify target regions for sequencing.

电话：400-614-0008
网站：www.kactusbio.cn

4.2 Perform Sanger sequencing and analyze knockout efficiency using SnapGene and EditR software. For precise quantification, submit samples for deep sequencing.

II. Protocol for 293T Cell Gene Knockout

1. Cell Preparation

1.1 Digest and plate cells when confluency reaches 70–90%.

1.2 Discard old medium and wash once with 5 mL 1× PBS.

1.3 Add trypsin to the flask, incubate at 37°C until cells detach, neutralize with 10% FBS-containing medium, resuspend into single-cell suspension, transfer to centrifuge tubes, and centrifuge at 300 × g for 5 min (RT).

1.4 Discard supernatant, resuspend in complete medium, and count using trypan blue exclusion.

1.5 Transfer 1E+06 cells per sample, centrifuge at 300 × g for 5 min (RT), discard supernatant, spin briefly at 100 × g for 2 min, and remove residual liquid with a 200 μL pipette.

2. Cell Electroporation

2.1 Reconstitute sgRNA dry powder with nuclease-free water to 2 μg/μL and store.

2.2 Prepare RNP complex: Mix 2.5 μg supra-ABE protein with 1.0 μg sgRNA, incubate at 37°C for 15 min.

2.3 Add 20 μL electroporation buffer (3.6 μL Solution 1 + 16.4 μL Solution 2) containing 1.0E+06 cells to the RNP mixture. Gently mix to avoid bubbles.

2.4 Transfer the mixture (≤30 μL) to a cuvette without bubbles.

2.5 Perform electroporation using the Lonza 4D-Nucleofector™: Select the X module, choose "293T cell" settings, and run program DS150. Remove cuvette and shut down the instrument.

2.6 Add 100 μL 1× dual-antibiotic medium to the cuvette, incubate for 15 min, transfer cells to a 24-well plate, rinse with 120 μL medium, adjust total volume to 1 mL, and culture.

3. Genomic Extraction and Sequencing Analysis

3.1 Observe cell morphology under a microscope and capture images after 24 h.

3.2 Harvest cells at 48 h for genomic DNA extraction. PCR-amplify target regions for sequencing.

3.3 Analyze editing efficiency via Sanger sequencing using SnapGene and EditR software. For high-accuracy results, submit samples for deep sequencing.

Notes

1. Avoid repeated freeze-thaw cycles

2. This product is intended solely for scientific research purposes and must not be used for any other applications

电话：400-614-0008
网站：www.kactusbio.cn