

GMP-Grade AccuBase™

Catalog #GMP-KD-0001

Product Information

Product name: BS-EP1 (AccuBase™)
Catalog #: GMP-KD-0001
Express System: *E. coli*
Production Requirement: Clean (Grade C or D)
Product Grade: GMP
Concentration 10 mg/mL
Purity(SEC-HPLC) ≥ 80%
Molecular Weight: 210.14kDa
Endotoxin: ≤ 10.0 EU/mg
Size: 1mg
Form: Liquid
Storage Buffer: 30 mM Tris, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol, pH8.0
Storage/Transport Transport on dry ice. Store at -80 ±10°C. Avoid repeated freezing and thawing.

Product Description: AccuBase™ is a genetically engineered DNA cytosine base editor (CBE) that efficiently edits DNAs within a range of 3-12 positions (with position No. 1 being farthest from the PAM site) (Figure 1). By integrating the deaminase into the Cas enzyme, AccuBase™ minimizes random binding of the enzyme to non-target DNAs and therefore can reduce off-target effects. AccuBase™ forms an RNP complex when combined with sgRNA (also compatible with the sgRNA for SpCas9). When the complex is introduced into cells and binds to the target site, it deaminates cytosine (C) into uracil (U) without causing DNA double-strand breaks. The uracil is then converted to thymine (T) via DNA repair. This C → T mutation can knock out target genes by generating a stop codon or disrupting the alternative splicing site.

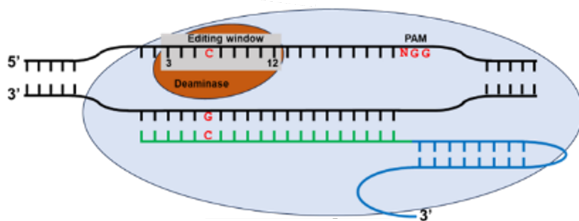


Figure 1. Editing window of AccuBase™

Applications

- Gene Knockout: The C → T mutation can knock out target genes by generating a stop codon or disrupting the alternative splicing site.
- Precise repair of defective genes harboring point mutations.
- Animal and plant breeding; Microinjection for Mouse or Zebrafish embryos.

Quality Specifications

Item	Acceptance Criteria
pH	8.0±0.5
Concentration	9.0-11.0mg/mL
Purity (electrophoresis)	≥ 80.0%
Purity (RP-HPLC)	≥ 88.0%
Purity (SEC-HPLC)	≥ 80.0%
Residual DNase	Sample/Control ≤ 3.0
Residual RNase	Sample/Control ≤ 3.0
Residual Host Cell Protein	≤ 100.0ng/mL
Residual Host Cell DNA	≤ 200.0ng/mL
Endotoxin	≤ 10.0EU/mg
Sterility	Negative
Mycoplasma	Negative

Product Validation

Identity: Bis-Tris PAGE

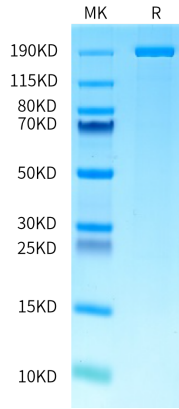


Figure 2. Molecular Weight of AccuBase™ is larger than 190 KDa with a clean single band by Bis-Tris PAGE.

Off-target Event Measurement

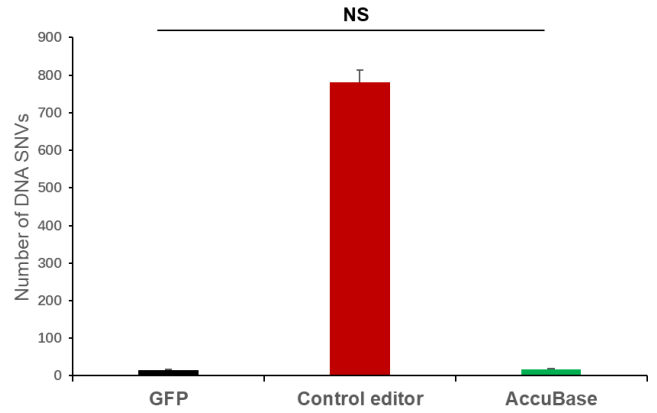


Figure 4. Measurement of off-target effects by GOTI. By leveraging the GOTI (genome-wide off-target analysis by teo-cell embryo injection) to measure the off-target effects throughout the whole genome, it was shown that compared to the control base editor (with 700 SNVs detected), the number of SNV obtained after editing by AccuBase™ is similar to the GFP (negative control) group, suggesting a near-to-zero off-target effects of AccuBase™.

Gene editing efficiency

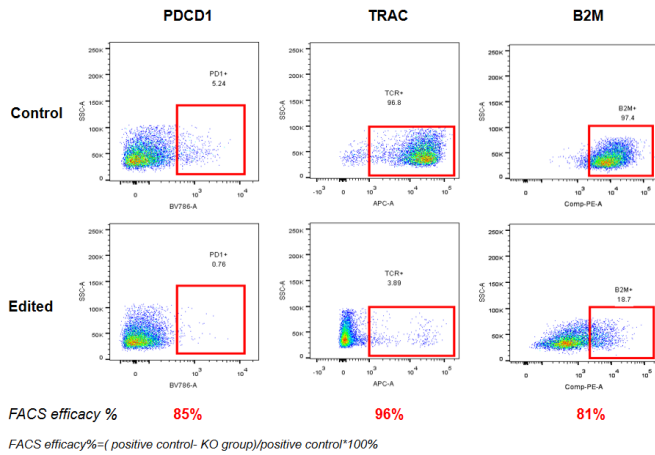


Figure 3. The PD1, B2M and TRAC protein levels on the T cell membrane are significantly decreased after gene editing by AccuBase™. The knockout efficiency for *PD1* and *B2M* with AccuBase™ is 85% and 81%, respectively, while for *TRAC* gene, the efficiency can reach 96%.

Purity (SEC-HPLC)

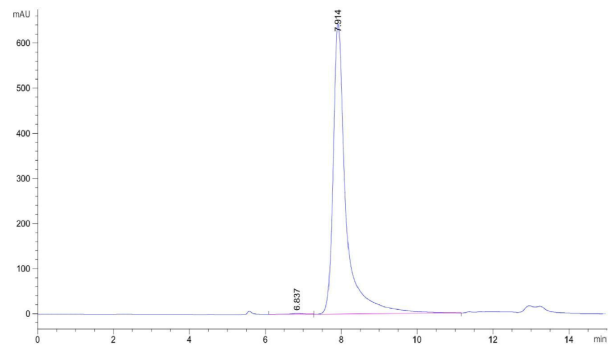


Figure 5. The purity of AccuBase™ is higher than 80% tested by SEC-HPLC assay.

Recommended Protocol

1. Cell Preparation (Example: T cell)
 - a. Prepare 1×10^6 cells for a 20uL transfection system.
2. Transfection of T cells with AccuBase™
 - a. Prepare electroporation buffer and pre-warmed cell culture medium
 - b. Centrifuge the required number (1×10^6) of cells at 90g for 10 minutes, then wash the cell pellet with 1X PBS once. Centrifuge again and remove the supernatant.
 - c. Resuspend cells with electroporation buffer at room temperature and mix cells with the required amount of AccuBase™ and sgRNAs. For 1×10^6 cells, the recommended amount of AccuBase™ and sgRNA is 80 pmol and 80 pmol, respectively. Transfer the mixture to the electrical cartridge. Avoid generating bubbles.
 - d. Follow the instructions of electroconverter to perform the transfection.
 - e. Transfer cells to the pre-warmed medium carefully.
 - f. Steps (C - E) need to be finished within 15 minutes.
3. Evaluation of gene editing efficiency
 - a. Check the gene editing efficiency 72 hours after transfection.
 - b. Option #1: Run FACS assay to analyze the ratio of cells positive of the target gene(s) within the cell population.
 - c. Option #2: Amplify target genes via PCR and perform sequence analysis.

Notes

1. Avoid repeated freezing and thawing.
2. Pre-mixing of AccuBase™ and sgRNA is not recommended before electroporation.

Additional Support

Contact us at support@kactusbio.us for additional questions or protocol support.