60 Hickory Drive
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AccuBase™

#KD-0001

Product Information

Product Name	Sizes
BS-EP1 (10mg/mL)	200μg / 1mg

Product Description

AccuBase™ (product name: BS-EP1) 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)(Fig 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.

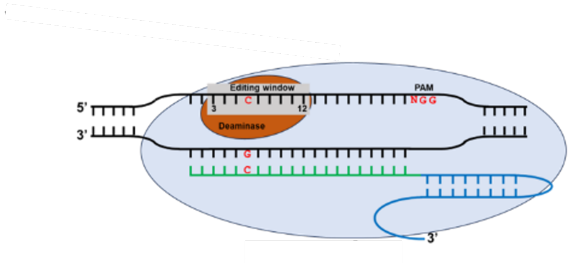

Fig 1. Editing window of AccuBase™

Source *E. coli*

Concentration 10 mg/mL

Purity(SEC-HPLC) ≥ 80%

Identity Molecular Weight larger than 190 KDa

Endotoxin ≤ 10.0 EU/mg

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 ±5°C. Avoid repeated freezing and thawing.

Application

1. Gene Knockout

The C->T mutation can knock out target genes by generating a stop codon or disrupting the alternative splicing site.

2. Precise repair of defective genes harboring point mutations

3. Animal and plant breeding; Microinjection for Mouse or Zebrafish embryos

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Product Validation

Identity

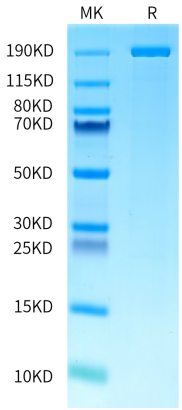


Fig 2. Molecular Weight of AccuBase™ is larger than 190 KDa with a clean single band by Bis-Tris PAGE.

Purity (SEC-HPLC)

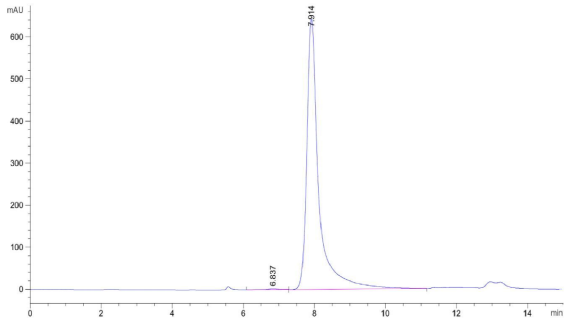
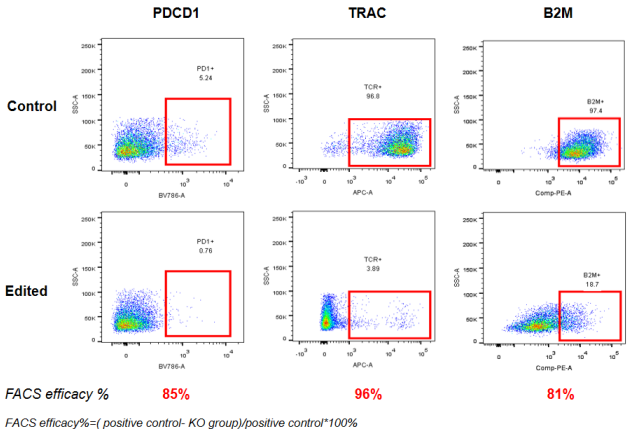


Fig 3. The purity of AccuBase™is higher than 80% tested by SEC-HPLC assay.

Gene editing efficiency



*FACS efficacy%=(positive control- KO group)/positive control*100%*

Fig 4. 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%

Notes

1. Avoid repeated freezing and thawing.
2. Pre-mixing of AccuBase™ and sgRNA is not recommended before electroporation
3. This product is for research only.

Protocol

1. Cell Preparation (Example: T cell)

Prepare 1x10^6 cells for a 20ul transfection system.

2. Transfection of T cells with AccuBase™

1) Prepare electroporation buffer and pre-warmed cell culture medium

2) Centrifuge the required number (1X10^6) of cells at 90g for 10 minutes, then wash the cell pellet with 1X PBS once. Centrifuge again and remove the supernatant.

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3) Resuspend cells with electroporation buffer at room temperature and mix cells with required amount of AccuBase™ and sgRNAs. For $1X10^6$ 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.

4) Follow the instructions of electroconverter to perform the transfection.

5) Transfer cells to the pre-warmed medium carefully.

Steps (3-5) need to be finished within 15 minutes.

3. Evaluation of gene editing efficiency

Check the gene editing efficiency 72 hours after transfection.

Option #1: Run FACS assay to analyze the ratio of cells positive of the target gene(s) within the cell population.

Option #2: Amplify target genes via PCR and perform sequence analysis