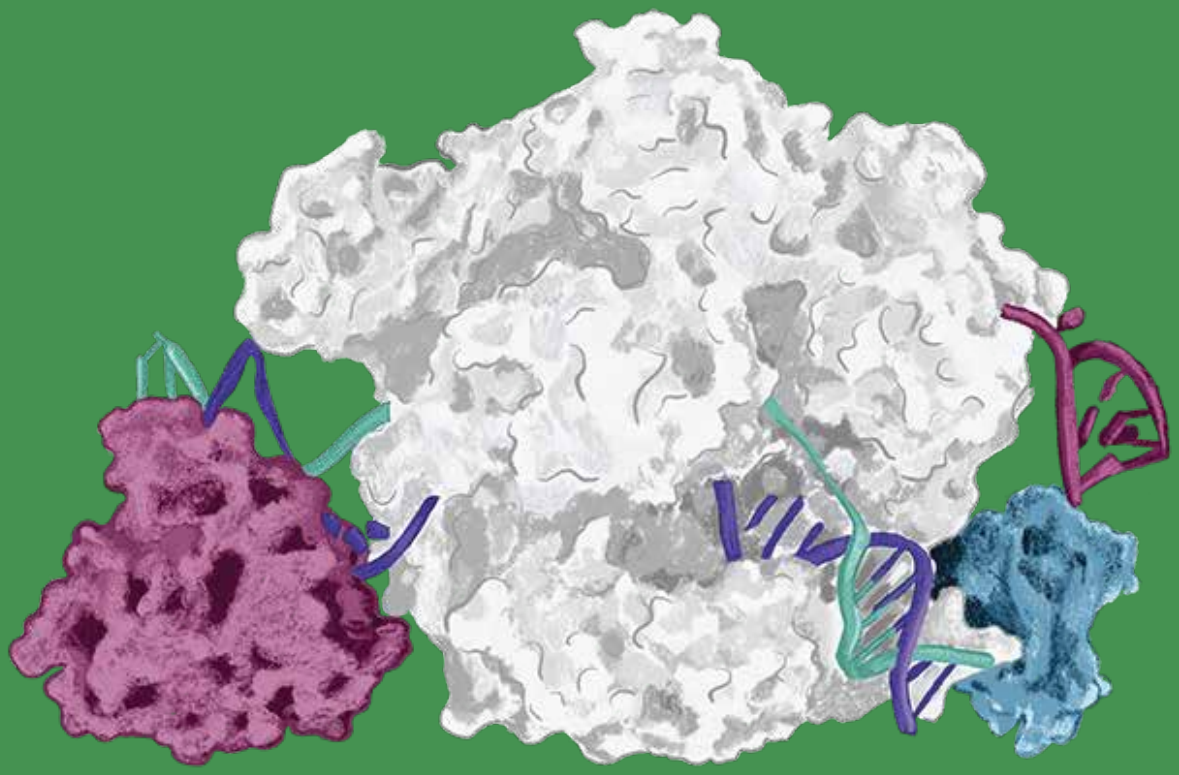
KACTUS

# AccuBase®
# Cytosine Base Editor

## GMP-Grade & Research-Grade

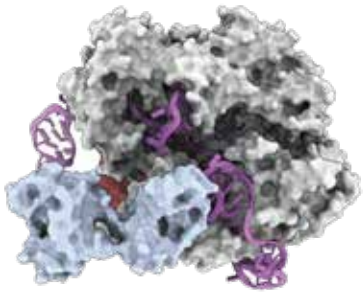


kactusbio.com
support@kactusbio.us

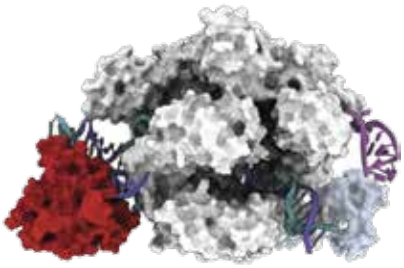
60 Hickory Drive
Waltham, MA 02451
United States

## About AccuBase® Base Editor

AccuBase® is a cytosine base editor engineered by Base Therapeutics and manufactured for commercialization by KACTUS. It creatively embeds a deaminase inside the Cas protein to prevent random binding of deaminase to non-target sites, significantly reducing off-target occurrence while still maintaining high editing efficiency. AccuBase® is a cytosine base editor which can convert a C-G base pair into a T-A base pair.



Non-editing Stage



Base-editing Stage

**Figure 1.** Structural image of AccuBase® in non-editing state and base-editing state.

After forming a ribonucleoprotein (RNP) with sgRNA, the AccuBase® protein remains in a non-editing state before binding to the target DNA *(Figure above, left)*. The deaminase is encapsulated inside the Cas9n protein and does not interact with any non-target DNA, thus significantly reduces the risk of off-target effects.

When the RNP binds to the target DNA, the conformation of AccuBase® changes, leading to the exposure of deaminase domain and effectively editing bases within the 3-12 window range of the target site (with the first position being the farthest from the PAM) *(Figure above, right)*.

## How does AccuBase® Base Editor work?

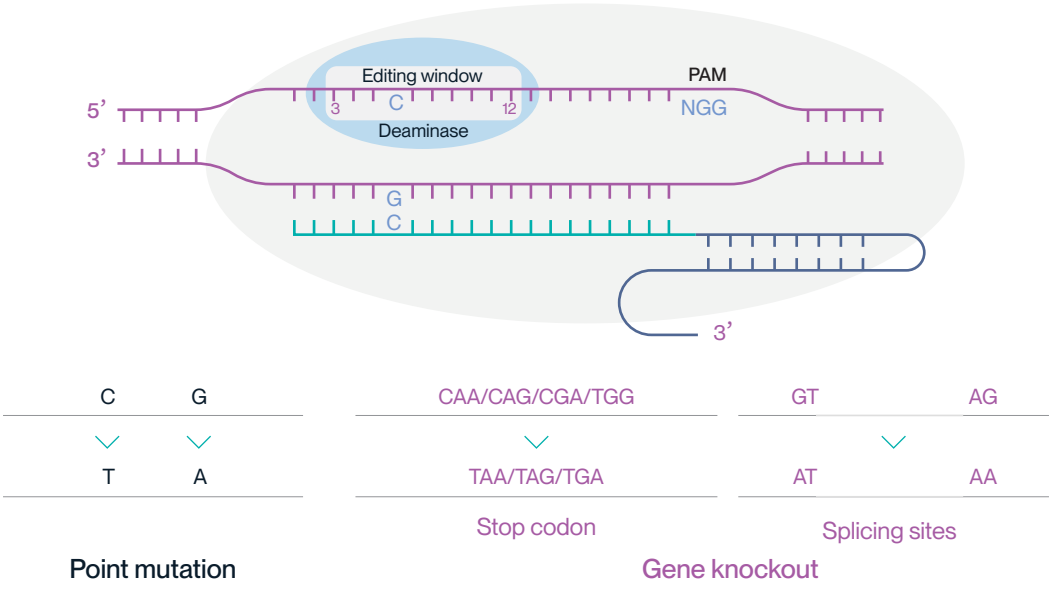



**Figure 2.** AccuBase® mechanism of action.

## AccuBase® Base Editor is ideally suited for:

**Precise Gene Correction:**

Correcting single point mutations to restore normal gene function.

**Targeted Gene Knockout:**

Silencing gene expression by introducing stop codons or disrupting splice sites.

**Multi-locus Editing and Compatibility:**

Editing multiple genes simultaneously. AccuBase® can also be combined with other gene editing tools.

# GMP Compliance

## Product Specifications

| Parameter | Specification |
|---|---|
| Express System | E. Coli |
| Concentration | 10mg/ml |
| Molecular Weight | 210.14kDa |
| Form | Liquid |
| Storage Buffer | 30 mM Tris, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol, pH8.0 |
| Storage | Store at -80 ±10°C |

## Regulatory Documentation

AccuBase®, GMP-Grade (GMP-KD-0001) comes with customizable regulatory documentation including **Data Sheet, MSDS, COA, TSE/BSE statement, CoO.** KACTUS has a comprehensive documentation system including a digital quality management system, specification program, criteria support documents, and quality reports. Our product release process involves 15+ contaminant detection steps, 30+ quality tests, 4+ report reviews, and 4+ product release checkpoints. We can customize your documentation package based on relevant regulatory filings.

## Quality Specifications for AccuBase®

AccuBase®, GMP-Grade (GMP-KD-0001) is manufactured according to cGMP guidelines in our 10,000m² manufacturing facility and undergoes the following quality release testing.

| Parameter | 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 |

# Why choose AccuBase® Base Editor?

## Near-Zero Off-Target Event Measurement

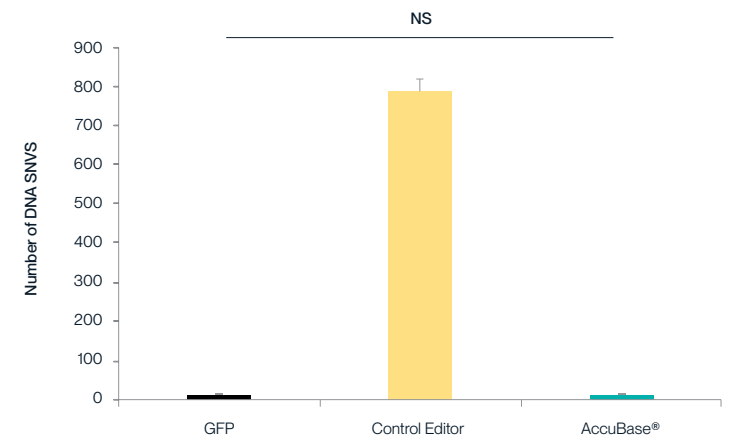



**Figure 3.** Measurement of off-target effects by GOTI. By leveraging the GOTI (genome-wide off-target analysis by teo-cell embryo injection) to measure the off-target effects throughout the whole genome, it was shown that compared to the control base editor (with 700 SNVs detected), the number of SNV obtained after editing by AccuBase® is similar to the GFP (negative control) group, suggesting a near-to-zero off-target effects of AccuBase® .

## Avoids creation of INDELs and chromosomal translocation

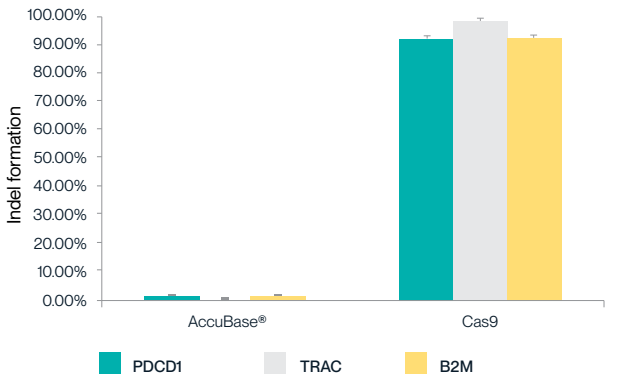



**Figure 4.** Compared with Cas9 protein, AccuBase® does not produce DNA horizontal insertions and deletions (INDELs).

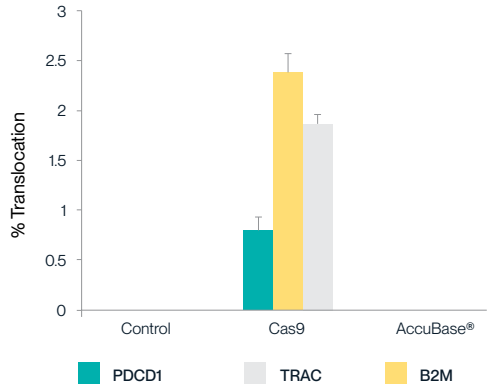
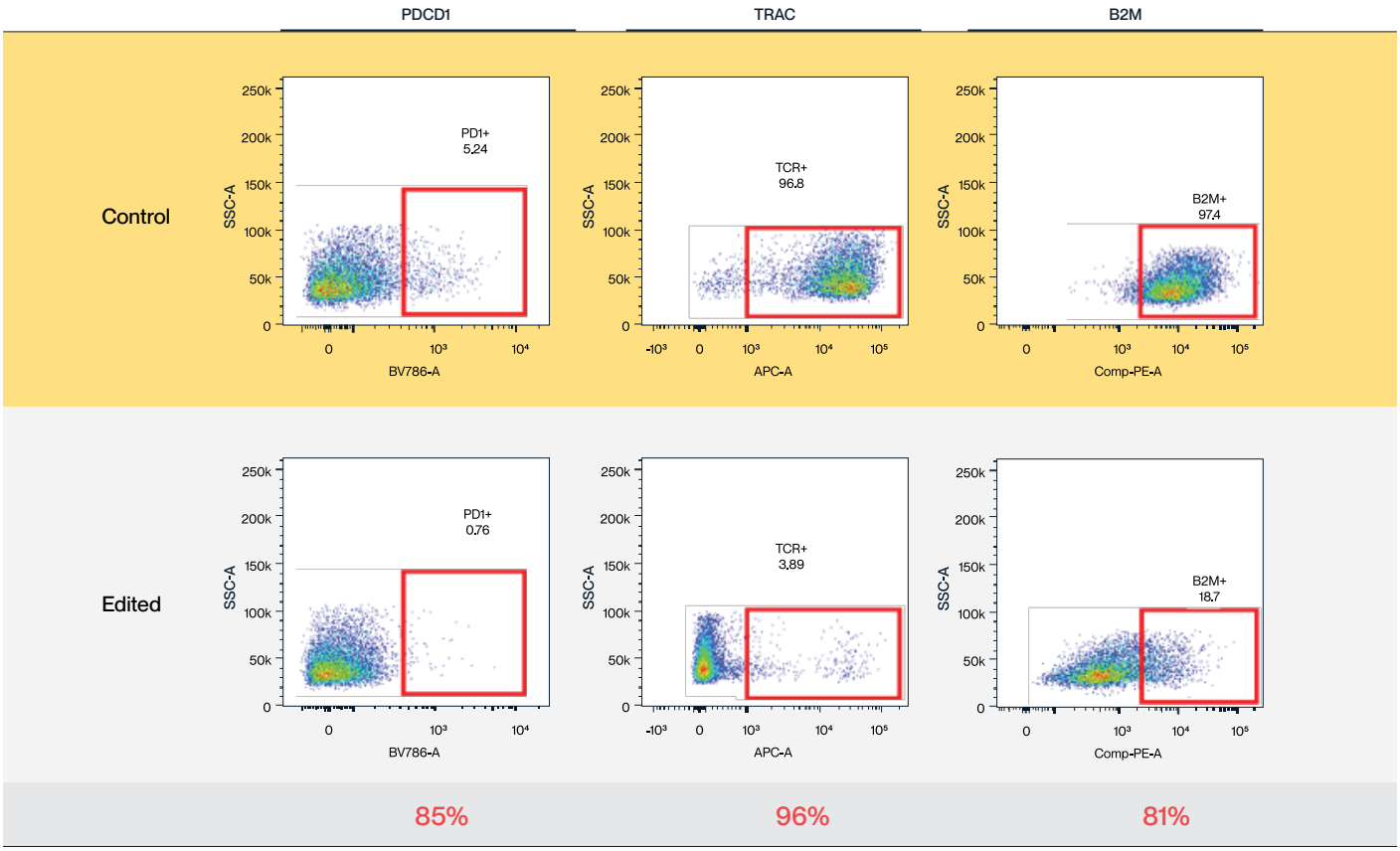



**Figure 5.** Compared with Cas9 protein, AccuBase® does not cause chromosomal translocation.

# High Base Editing Activity

## Activated Primary T Cells




**FACS efficacy %**

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

**Figure 6.** AccuBase® RNP was electroporated into activated primary T cells. According to flow cytometry, AccuBase® can efficiently knock out PD1, B2M, and TRAC proteins on the membrane of activated primary T cells at the protein level. For PD1 and B2M, the knockout efficiency exceeded 80%, while the knockout efficiency of TRAC reached 96%.

## Unactivated Primary T Cells

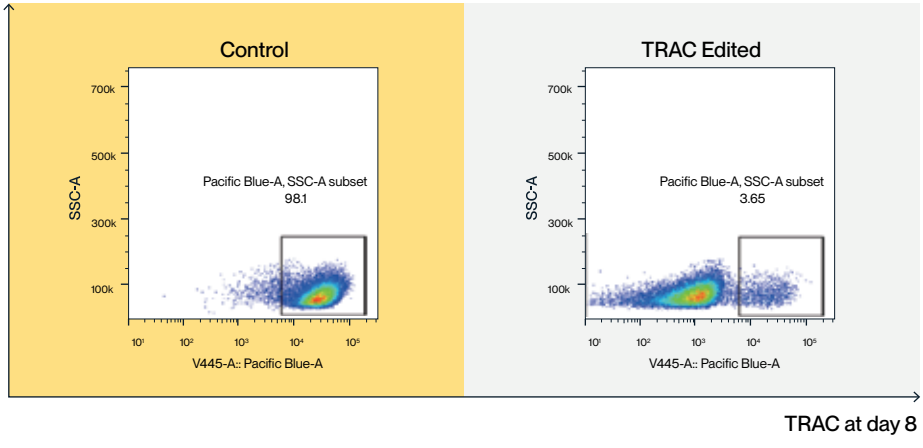



**Figure 7.** AccuBase® RNP was electroporated into activated primary T cells. According to flow cytometry, AccuBase® can efficiently knock out the TRAC protein of unactivated primary T cells at the protein level, with a knockout efficiency of up to 96%.

## AccuBase® Base Editor Features

GMP

Manufactured in a GMP-compliant facility

Raw materials free from animal-derived components

High purity, activity, and stability

Traceable documentation for regulatory support

Off-the-shelf catalog product

## AccuBase® ELISA Kit

We have developed an AccuBase® ELISA kit for detection of residual AccuBase® in samples. The kit uses monoclonal antibodies in a sandwich ELISA and demonstrates high accuracy.

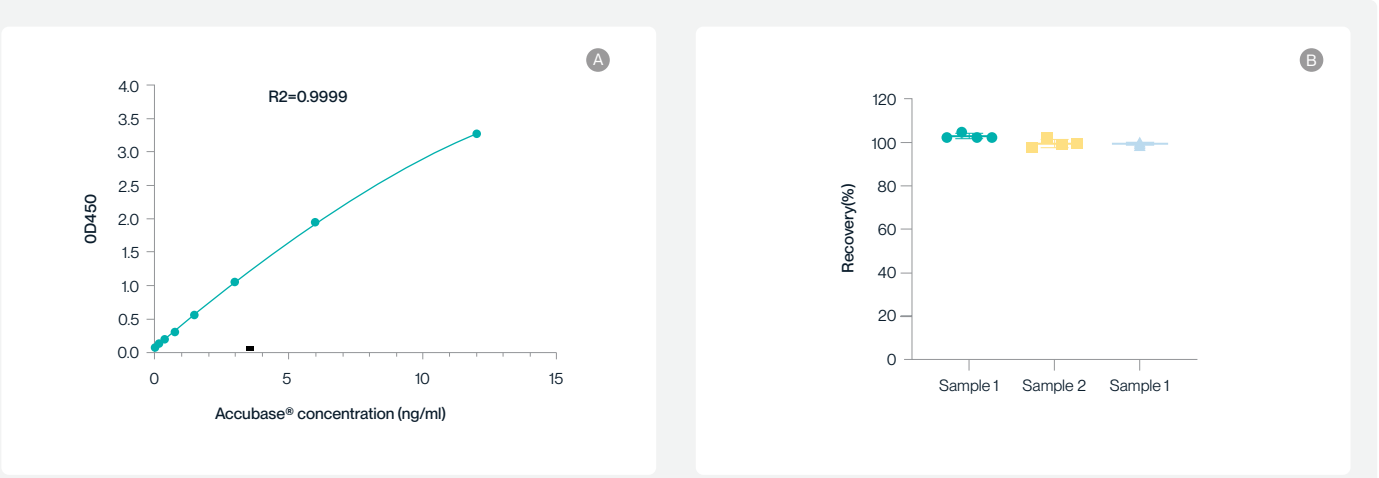



**Figure 8.** (A) Example 8-point standard curve for AccuBase® ELISA. The quantitative range of this ELISA kit is 187.5 pg/mL - 12 ng/mL. (B) The recovery rate of the kit was analyzed by testing three AccuBase® samples with known concentrations in dilution buffer using the same AccuBase® ELISA kit. Results show that in dilution buffer, the recovery rate of various AccuBase® sample concentrations is between 80% and 120%, indicating that this reagent kit has high accuracy.

### Assay Performance

| | |
|---|---|
| **Detection range:** | 0.1875ng/ml-12 ng/ml |
| **Sensitivity:** | 0.1 ng/ml |
| **Accuracy:** | CV<10% |

# Frequently Asked Questions

## Product Specifications

### Is AccuBase® manufactured according to GMP guidelines?

Our GMP-Grade AccuBase® (#GMP-KD-0001) is always manufactured according to cGMP guidelines. We also offer a research-grade version of AccuBase® (#KD-0001) that does not undergo GMP quality control testing.

### What regulatory documentation is available for GMP-Grade AccuBase®?

AccuBase®, GMP-Grade (#GMP-KD-0001) comes with customizable regulatory documentation including Data Sheet, MSDS, COA, TSE/BSE statement, and CoO. Batch production records, batch inspection records, etc, can also be provided. Please contact us so we can learn more about your specific regulatory filings.

### What are the advantages of AccuBase® over other gene editing enzymes?

Compared to Cas9 and other Cas family gene editing enzymes, AccuBase® does not cause DNA double-strand breaks, does not require donor DNA, and is not dependent on the cell cycle for precise gene repair. It also does not produce INDELs and thus poses no risk of chromosomal translocations.

Compared to other CBEs (Cytosine Base Editors), its unique structural design allows editing only at the target site, with no random off-target activity, while maintaining high editing efficiency.

### What is the difference between research-grade and GMP-grade AccuBase®?

- **Production Environment.** GMP-grade AccuBase® is produced in our GMP manufacturing facilities, whereas research-grade is produced in non-GMP facilities.
- **Quality Control Testing.** GMP-grade AccuBase® includes tests for appearance, concentration, purity (2100), purity (SEC-HPLC), purity (RP-HPLC), endotoxin, DNA enzyme residue, RNA enzyme residue, host protein residue, host DNA residue, sterility, and mycoplasma. Research-grade includes concentration, identification (Bis-Tris PAGE), purity (SEC-HPLC), and endotoxin.
- **Regulatory Documentation.** GMP-grade AccuBase® includes complete method validation, process validation reports, COA, COO, non-animal source statements, stability studies, etc., supporting client project applications. Research-grade includes instruction manuals and COA, not supporting client project applications.
- Both research-grade and GMP-grade have the same bacterial strain origin, same materials used in production, same production process, and consistent protein activity.

## Product Usage

### Can AccuBase® Achieve Multi-Gene Knockout?

AccuBase® can not only achieve single-gene knockout but also simultaneously knock out 2, 3, or more genes.

### How do I design sgRNA for AccuBase®?

The single guide RNA (sgRNA) scaffold for AccuBase® is the same as that used for spCas9. Designing sgRNA for gene knockout with AccuBase® involves considering editing within the 3-12 position window. A simple approach is to first search for triplet codons CAA (Gln), CAG (Gln), CGA (Arg), or TGG (Trp), and then look for suitable PAMs (NGG) nearby.

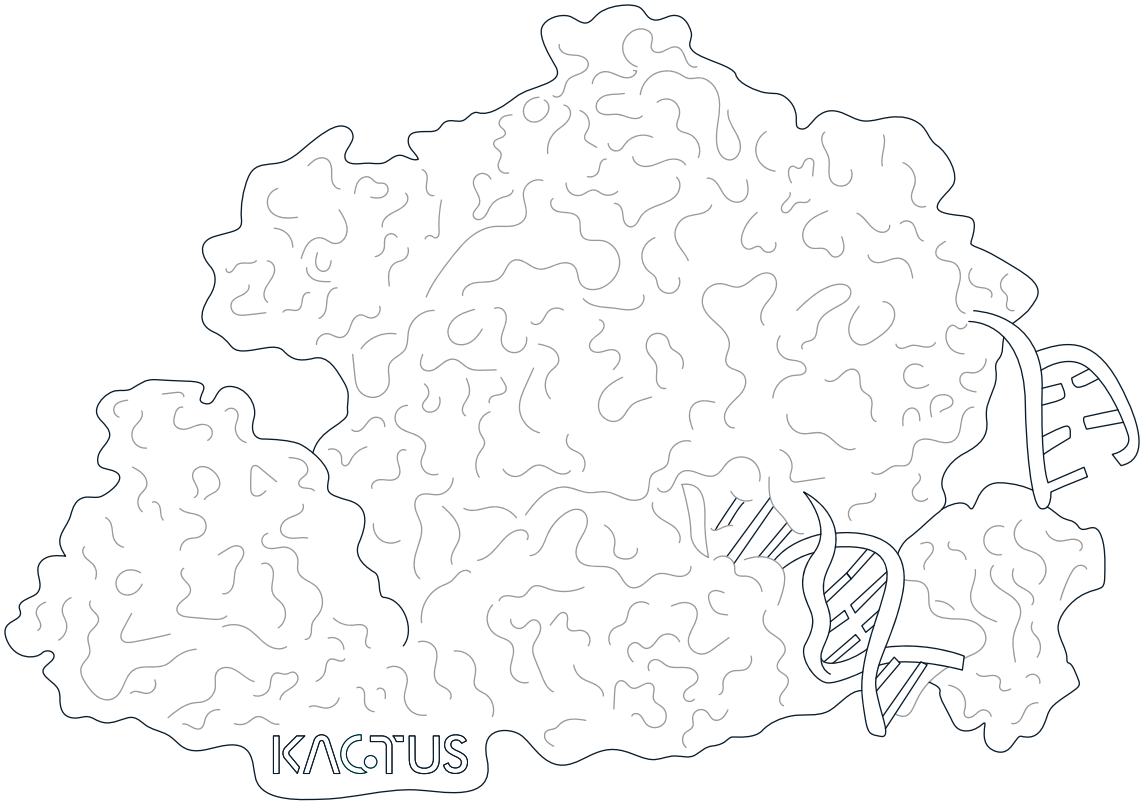
## Ordering Information

| Catalog # | Product Name | Grade | Available Sizes |
|---|---|---|---|
| KD-0001 | AccuBase®, BS-EP1, RUO | Research-Grade | 200µg / 500µg / 1mg |
| GMP-KD-0001 | AccuBase®, BS-EP1, GMP | GMP-Grade | 1mg |
| RD-K-014 | BS-EP1 (AccuBase®) ELISA Kit | For research use only | 96 Tests |

**Note:** Accubase® is the trade name of BS-EP1.

## Request a Quote or More Information

Please contact support@kactusbio.us to request a quote or additional information for our AccuBase® Base Editor. One of our team representatives would be happy to speak with you!




Learn More