High-Performing GMP CRISPR Cas9 Protein for Cell and Gene Therapy

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Introduction

CRISPR Cas9 is the leading technology for gene editing and has been widely applied in advanced therapeutic research. To fulfill the need for high-quality and high-performing Cas9 protein for ex vivo cell therapy drug manufacturing, KACTUS has successfully produced GMP-grade Cas9 enzyme in compliance with cGMP regulations. This enzyme undergoes strict and comprehensive quality control and management. KACTUS GMP-grade Cas9 enzyme has demonstrated good batch-to-batch consistency and long-term stability for purity and activity. Compared to a leading commercial Cas9 enzyme, KACTUS GMP-grade Cas9 enzyme showed comparable gene editing efficiency in both in vitro cleavage assay and in multiple cellular gene editing. Currently, KACTUS GMP-grade Cas9 protein has been successfully applied in multiple clinical manufacturing processes of ex vivo cell therapy drug products, with proven track records for supporting investigational new drug (IND) filings worldwide.

Objective

The primary goal was to demonstrate the capability of KACTUS in manufacturing GMP-grade Cas9 enzyme with high yield, consistent batch-to-batch purity and activity, as well as substantial long-term stability. This goal was underpinned by the need to minimize risks and align with compliance standards. Achieving these goals was essential for maintaining the reliability and effectiveness of the gene editing processes involved, providing a solid foundation for operational excellence.

Methodology

KACTUS leveraged our well-established process development, manufacturing and analytical teams to ensure the delivery of GMP-grade Cas9 enzyme with high quality and performance. Technically, the process development team designed the proprietary nuclear localization signal (NLS) and optimized the expression and purification process to ensure high yield and activity. During manufacturing, the use of animal origin-free materials and the implementation of a closed process under Good Manufacturing Practice (GMP) environment guaranteed minimal quality risks and compliance to GMP regulation. Finally, methods for quality control were developed and qualified in-house, ensuring strict quality control (QC) testing before release.

Quality Control Specifications

Parameter	Criteria
Concentration	9.5-11.5mg/mL
Purity (RP-HPLC)	≥95%
Purity (SEC-HPLC)	≥95%
Activity (In Vitro Cleavage)	>85%
Endotoxin	≤10EU/mg
Residual DNase	≤LOD
Residual RNase	≤LOD
Residual Host Protein	≤100ng/mL
Residual Host Cell DNA	≤3ng/mL
Sterility	Negative

Robust & High-Quality Production

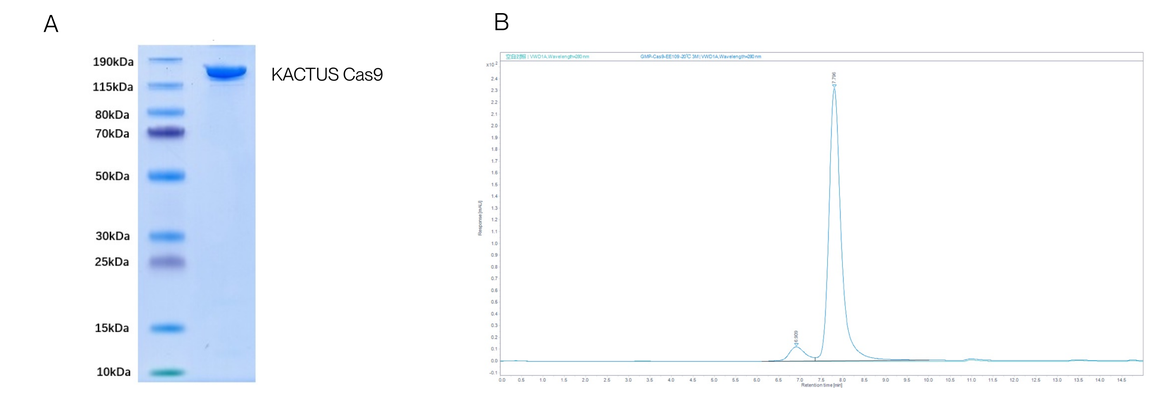

Figure 1. A high purity Cas9 protein was obtained based on the KACTUS GMP production process. (A) Detection by Tris-Bis-PAGE. (B) Analysis by SEC-HPLC (Agilent). Results show the purity is greater than 95%.

Excellent Long-Term Stability and Batch-to-Batch Consistency

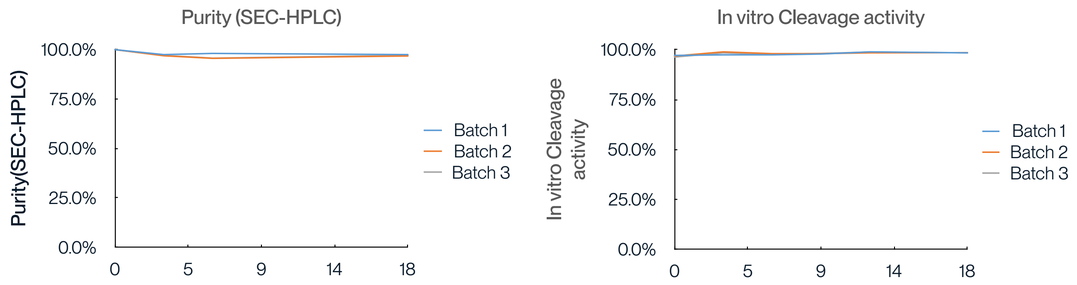

Figure 2. Consistent high purity and activity performance across three manufacturing batches of KACTUS GMP-grade Cas9. Three batches of KACTUS GMP-grade Cas9 were stored at -20°C for stability study, assessing the purity by SEC-HPLC and in vitro cleavage activity at 3, 6, 9, 12, 15, 18 months, respectively. The results showed KACTUS GMP-grade Cas9 has good stability and high consistency among different manufacturing batches.

High In Vitro Cleavage Activity

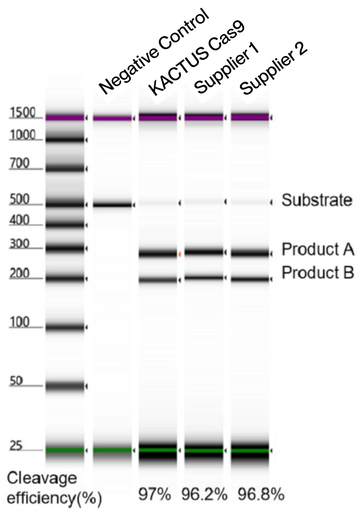

Figure 3. KACTUS GMP-grade Cas9 shows a comparable in vitro cleavage activity with Cas9 from other leading suppliers. 2μL (0.1μg/μL) of Cas9 protein was mixed with 2μL of 2μM sgRNA to cut 300ng substrate DNA for 15min at 37°C. Then, 4150 TapeStation was used to analyze the cleavage efficiency. KACTUS GMP-grade Cas9 achieved cleavage efficiency of 97% vs 96.2% for supplier 1 and 96.8% for supplier 2. (N=3)

High Gene Editing Efficiency Across Multiple Cell Types

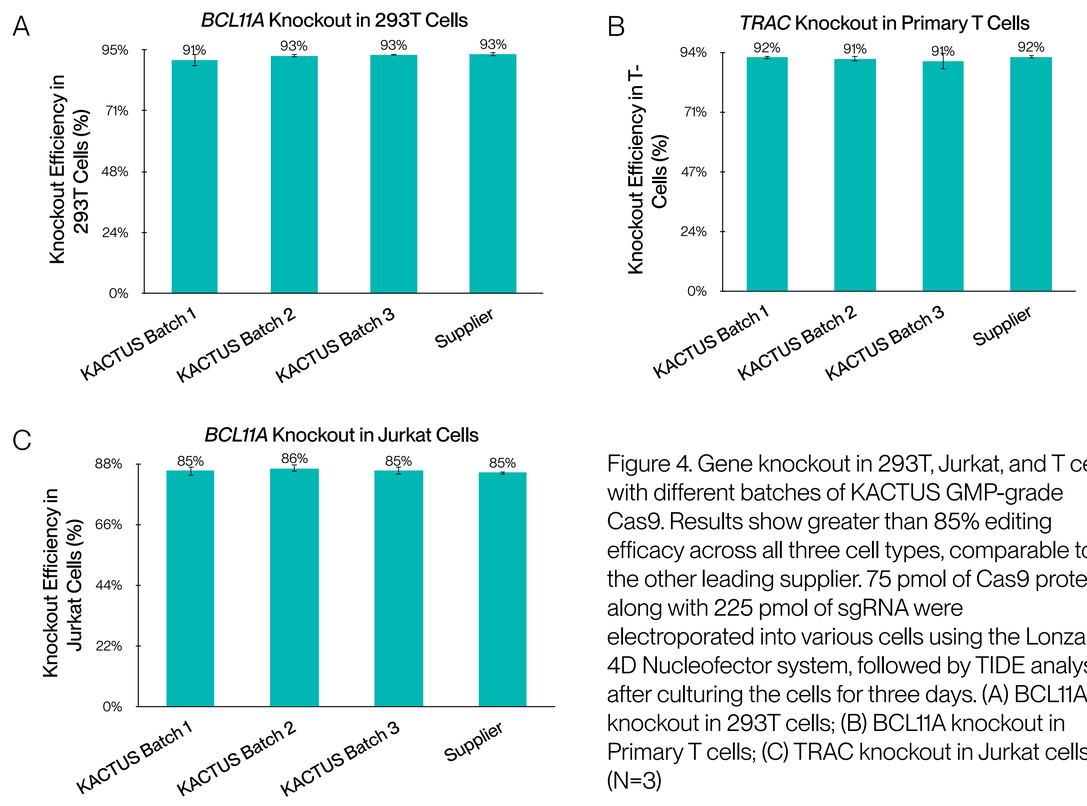

Figure 4. Gene knockout in 293T, Jurkat, and T cells with different batches of KACTUS GMP-grade Cas9. Results show greater than 85% editing efficacy across all three cell types, comparable to the other leading supplier. 75 pmol of Cas9 protein along with 225 pmol of sgRNA were electroporated into various cells using the Lonza 4D Nucleofector system, followed by TIDE analysis after culturing the cells for three days. (A) BCL11A knockout in 293T cells; (B) BCL11A knockout in Primary T cells; (C) TRAC knockout in Jurkat cells. (N=3)

Gene Editing in Primary T cells for Allogenic CAR-T

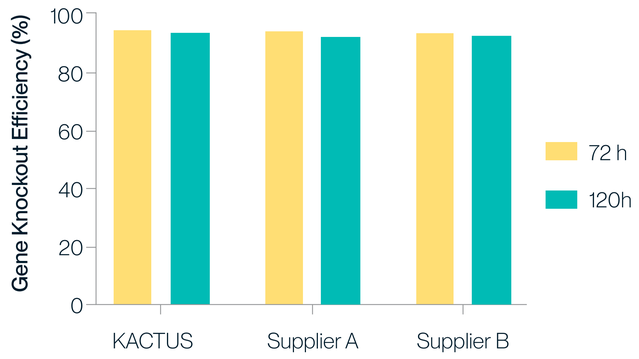

Figure 5. Gene knockout of the HLA gene for Allogenic CAR-T therapy. KACTUS GMP-grade Cas9 demonstrates efficient gene editing activity, comparable to two other suppliers in the market.

Gene Editing in Human Stem Cells (HSCs) for β-thalassemia

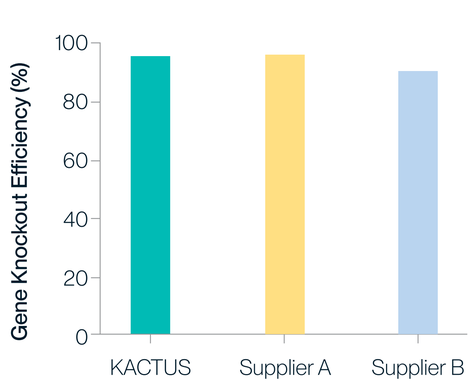

Figure 6. KACTUS GMP-grade Cas9 shows efficient BCL11A knockout activity in gene editing human stem cells (HSCs) for the treatment of β-thalassemia. The knockout efficiency is comparable to two other leading suppliers in the market.

Universal High Sensitivity ELISA Kit for Quantitative Detection of Cas9 Residues

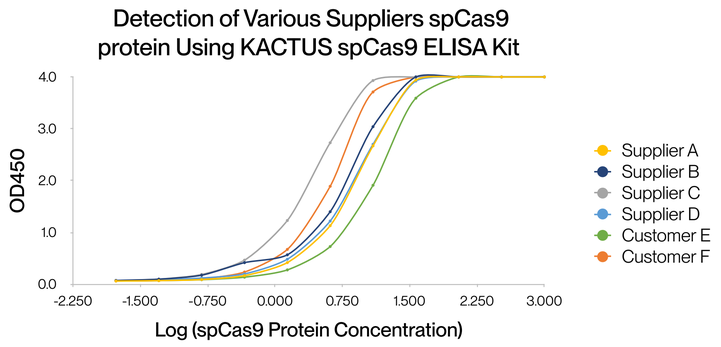

Figure 7. KACTUS residual spCas9 detection ELISA kit (sandwich ELISA method) demonstrates high efficiency in quantitatively detecting spCas9 enzymes from various suppliers, suggesting its potential as a universal spCas9 detection kit.

Off-the-Shelf Product List

KACTUS has developed the following off-the-shelf Cas9 enzymes and Cas9 ELISA kit to facilitate preclinical and clinical gene editing studies.

Catalog No.	Product Description
GMP-CAS-EE109	Cas9 Protein (GMP-Grade)
CAS-EE109	Cas9 Protein (Research-Grade)
CAS-MM00B	Cas9 (CRISPR Associated Protein 9) ELISA Kit

Conclusion

KACTUS demonstrated strong manufacturing capability in producing GMP grade Cas9 enzyme as critical raw materials for CGT drug product manufacturing. Our Cas9 protein features:

→ Animal origin-free manufacturing process and quality control release in strict compliance with GMP regulations.

→ Outstanding enzyme activity validated in both in vitro cleavage and ex vivo gene editing in multiple cell lines.

→ Consistent batch-to-batch performance and excellent long-term stability.