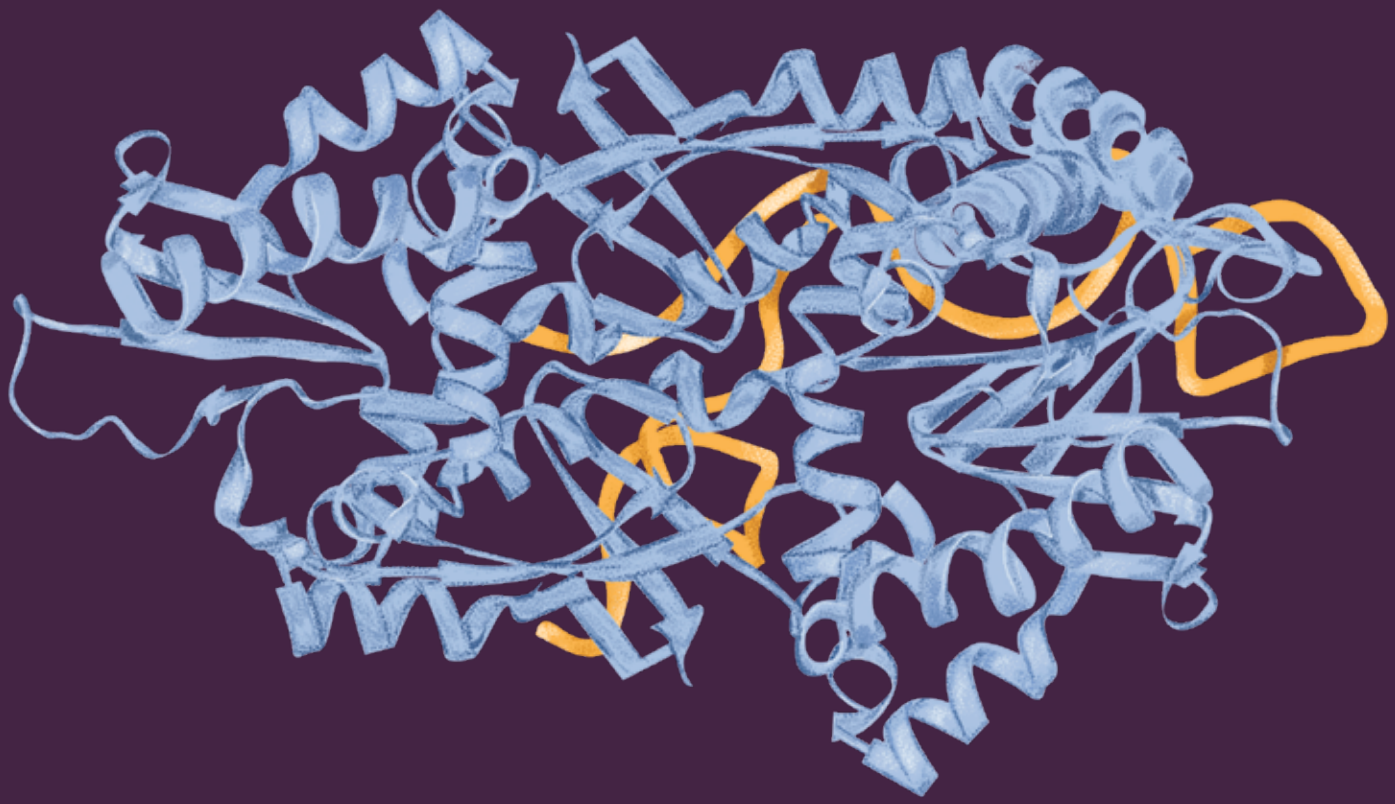
KACTUS

Cas9 Enzyme

GMP-Grade & Research-Grade



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GMP-Grade Cas9 Enzyme

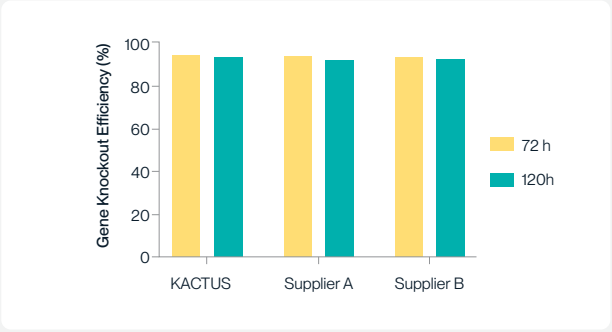
Catalog No. #GMP-CAS-EE109

KACTUS GMP-grade spCas9 is a top-performing CRISPR Cas9 protein, designed to achieve better ribonucleoprotein (RNP) editing efficiency. It is produced under cGMP conditions to meet the standards of Ancillary Materials for Cell, Gene, and Tissue-Based Products. It undergoes rigorous quality control to meet the needs of CGT development and clinical research.

Key Features:

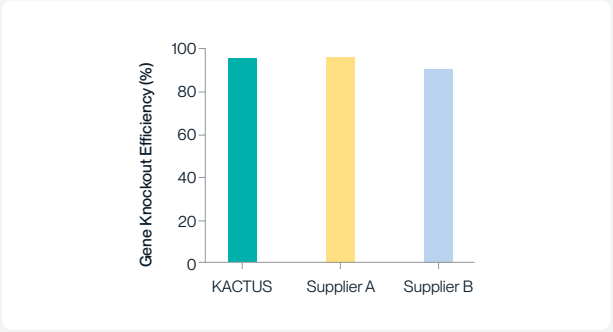
- **Wild-Type spCas9:** *Streptococcus pyogenes* Cas9 protein, engineered and expressed in *E. coli*
- **High Editing Efficiency:** Proven performance across multiple cell types, including primary T cells
- **Regulatory Support:** FDA DMF Type II filing to ease clinical applications
- **Streamlined Workflow:** Seamlessly transition from research-grade to GMP-grade
- **Proven Quality:** Supporting multiple FDA IND filings with leading biopharmaceutical companies

High Editing Activity



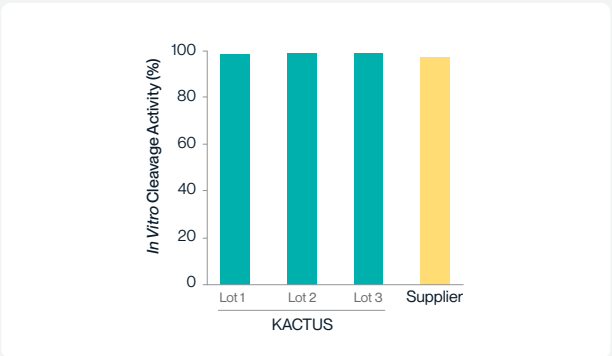
High Gene Knockout in Allogeneic CAR-T

Gene knockout was performed on CAR-T cells on genes related to GvHD and HvGR. KACTUS Cas9 nuclease and two leading suppliers were compared, with the gene knockout efficiency tested at 72h and 120h after electroporation. The performance of KACTUS Cas9 nuclease is comparable to that of leading suppliers.



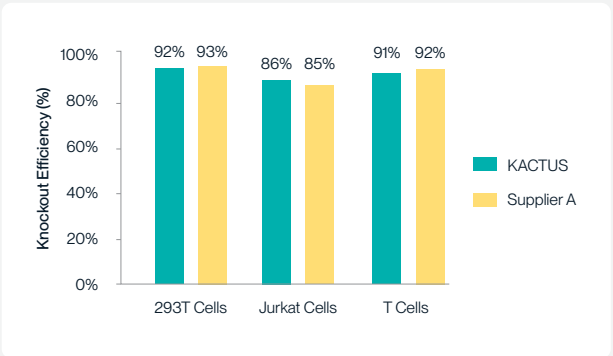
High Gene Knockout in HSCs

Gene knockout was performed on hematopoietic stem cells on genes related to β-thalassemia. KACTUS Cas9 nuclease and two leading suppliers were compared. The performance of KACTUS Cas9 nuclease is comparable to that of leading suppliers.



High *In Vitro* Cleavage Activity

KACTUS spCas9 activity is assessed using an *in vitro* cleavage assay. The results indicate that the activity exceeds 85% and is consistent across different batches.



High Editing Efficacy in Multiple Cell Types

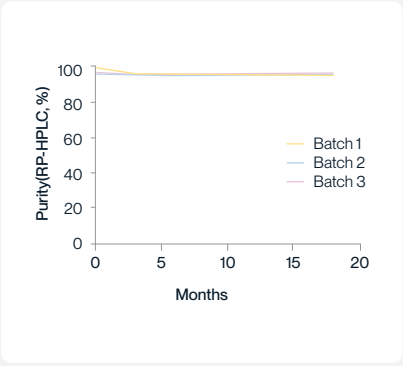
Gene knockout efficiency was analyzed in nucelofected 293T, Jurkat, and T cells using TIDE analysis. Results show > 85% editing efficacy across all three cell types, comparable to a leading supplier.

Quality Control Standards for GMP Cas9 Enzyme

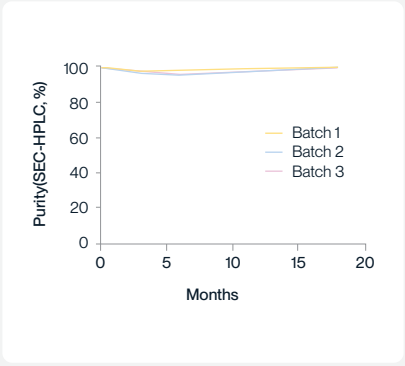
Catalog No. #GMP-CAS-EE109

Parameter		Acceptance Criteria
Appearance		Clear Liquid
Loading Amount		Not less than the amount of identification
Concentration		9.5-11.5mg/mL
Identification		Corresponding to Reference Standard
Purity	RP-HPLC	≥ 95.0%
	SEC-HPLC	Monomer ≥ 95.0%, Aggregates ≤ 5.0%
	NR-CE	≥ 85.0%
	R-CE	≥ 90.0%
Bacterial Endotoxin		≤10.0 EU/mg
Activity		≥85.0%
Residual DNase		Negative
Residual RNase		Negative
Residual Host Cell Protein		≤ 100.0 ng/mL
Residual Host Cell DNA		≤ 3.0 ng/mL
Sterility		No growth
Residual Nickel Salt		≤ 10.0 ppm
pH		7.4±0.5

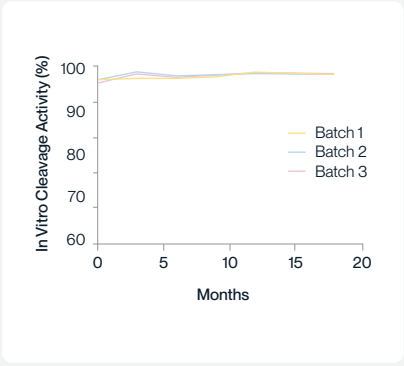
GMP Cas9 Enzyme is Stable Long-Term



RP-HPLC



SEC-HPLC



In vitro cleavage activity

Long-term stability data (0-18 months) of three batches of GMP-Grade Cas9 nuclease (#GMP-CAS-EE109). As shown in the figure, RP-HPLC and SEC-HPLC are both higher than 95%, indicating that the purity of the product performs well within 18 months; In addition, the *in vitro* cleavage activity showed no obvious downward trend within 18 months, and the activity was stable.

Universal spCas9 Nuclease ELISA Kit (#CAS-MM00B)

KACTUS has carefully developed a highly sensitive spCas9 detection kit (#CAS-MM00B).

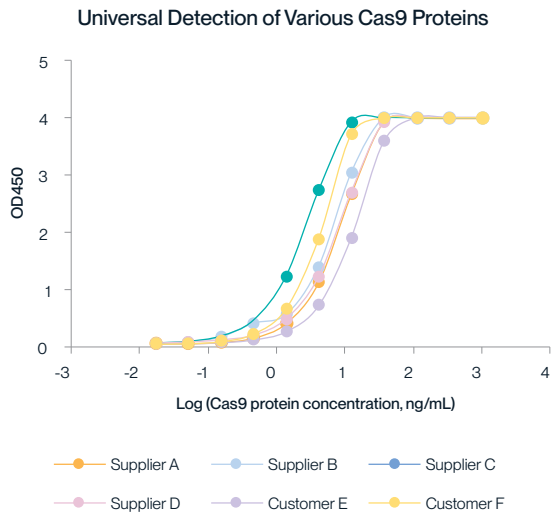
Applications:

- Detection of spCas9 protein residue
- Detection of spCas9 protein expression
- Universal spCas9 detection kit

Performance Specifications:

Detection range:	Sensitivity:	Accuracy:
0.25 ng/mL – 16 ng/mL	0.125 ng/mL	CV <10%

Product Data



Detection of various suppliers Cas9 protein using KACTUS Cas9 ELISA Kit. The Cas9 products were serially diluted 3-fold from 1000ng/mL and tested following the KACTUS Cas9 ELISA kit procedure. Results show that the KACTUS Cas9 ELISA kit can be used for the quantification of various vendors' Cas9 protein. The sensitivity for other vendors was 0.2-0.4ng/mL, which is close to the sensitivity for KACTUS Cas9 protein (0.125ng/mL).

The recovery rates of three different concentrations of Cas9 nuclease samples in dilution buffer ranged from 80% to 120%, indicating that the kit has high accuracy in detecting Cas9 nuclease.

Consistency

Sample Name	Batch	Number of Replicates	Average Detection Value M (ng/mL)	Coefficient of Variation CV (%)
1 (10ng/mL)	Batch 1	n=10	9.91	1.86%
	Batch 2	n=10	10.64	1.62%
	Batch 3	n=10	10.14	2.30%
2 (2.5ng/mL)	Batch 1	n=10	2.49	1.37%
	Batch 2	n=10	2.47	2.11%
	Batch 3	n=10	2.58	2.58%
3 (0.625ng/mL)	Batch 1	n=10	0.61	2.13%
	Batch 2	n=10	0.58	1.63%
	Batch 3	n=10	0.59	3.10%

A single batch of Cas9 ELISA kit was used to detect Cas9 protein samples with different concentrations. In addition, three batches of kits were tested to assess consistency and reliability.

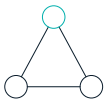
GMP Quality Management System

KACTUS has established a mature quality management system (QMS) and developed comprehensive regulatory documentation in accordance with pharmacuetical Good Manufacturing Practice (GMP) and ISO13485:2016 requirements. Our comprehensive documentation programs undergo continuous updates and improvements to ensure the effectiveness, appropriateness, and adequacy of our quality management system. Quality control is strictly managed at every production stage including raw and auxiliary material inspection, equipment validation, cell strain management, process development and optimization, analytical method development and validation, product packaging, and batch release testing. KACTUS' quality testing system ensures batch consistency and long-term stability so that our products meet the stringent requirements of drug manufacturing.

KACTUS Quality Management System and GMP facilities have passed audits and successfully supported multiple leading biopharmaceutical companies in completing FDA Investigational New Drug (IND) applications using our GMP-Grade Cas9 enzyme.

Cell Strain Control

Cell strains are strictly controlled, divided into Master Cell Bank (MCB) and Working Cell Bank (WCB).

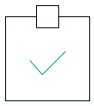


Analytical Method Verification

The analysis method is verified/confirmed by the system to ensure the validity and repeatability of the results.

Free from Animal- Derived Materials

The production process does not use raw and auxiliary materials containing animal sources, equipment, and facilities.

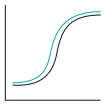


Quality Release Testing

Perform QC testing and release in all directions from central control samples, raw solutions, semi-finished products, and finished products.

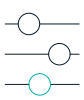
Continuous Process Improvement

The process is continuously improved and optimized to become more rational, stable, and feasible.



Batch-to-Batch Consistency

Continuously monitor batch-to-batch differences to ensure batch-to-batch consistency.



Control of Production Variables

The key parameters in the production process are strictly controlled to ensure consistency of products between batches.

Stability Testing

Continuous research on product stability, including influencing factors, tests, accelerated tests, long-term stability studies.

State-of-the-art GMP Manufacturing Facility

KACTUS operates a large GMP-certified manufacturing facility complete with large-scale protein expression and purification systems.

500L Dosing and Cleaning In Place (CIP) System

1000L Fermentation Tank

Aseptic filling

Chromatography System

Comprehensive Suite of Analytical Equipment

KACTUS has a comprehensive in-house portfolio of validated analytical equipment. Our verified analytical methods ensure accurate and reliable testing.

CytoFLEX SRT Flow Sorter

Biacore™ T200

1260 High-Performance Liquid Chromatograph

Multifunctional Microplate Reader

Capillary Electrophoresis Instrument

Frequently Asked Questions

What are the main differences between research-grade Cas9 enzyme and GMP-grade Cas9 enzyme?

Production Environment: GMP-Grade Cas9 is produced in a high-standard GMP-certified manufacturing facility. Research-grade Cas9 is produced in a standard manufacturing facility.

Quality Control: GMP-Grade quality control testing is more comprehensive than research-grade. Our GMP-Grade Cas9 undergoes 14 separate quality control tests before release.

Documentation Support: GMP-Grade Cas9 includes a customizable documentation package including Datasheet, TSE/BSE Statment, COA, COO, MSDS, DMF, Melamine Statement, and Nitrosamine Statement. Batch production records and batch inspection records can also be provided.

What methods are used to detect the activity of KACTUS Cas9 Enzyme?

We use two types of activity assays to analyze activity: in vitro cleavage activity and ex vivo knockout efficiency. Our in vitro cleavage activity assay assesses the cleavage activity of Cas9 by detecting the total mass ratio of two cleaved fragments formed and is a quality control release assay. Our ex vivo gene knockout efficiency was verified in 293T cells, Jurkat cells, and T cells. However, we do not verify the gene knockout efficiency of every Cas9 batch.

What is the knockout efficiency of KACTUS Cas9 Enzyme?

We have analyzed gene knockout efficiency of KACTUS Cas9 enzyme versus leading suppliers in 293T, Jurkat, and primary T cells. Our GMP Cas9 had a very high gene knockout efficiency, and is also on par with leading suppliers. However, the knockout efficiency depends on the cell type, target sequence, molar ratio of Cas9 to sgRNA, concentration of Cas9 to sgRNA, electroporator type, electroporation parameters, and other factors. We highly recommend testing out the enzyme in various conditions based on your project. Please reach out to us at sales@kactusbio.us to request a test sample.

Can the Cas9 (CRISPR Associated Protein 9) ELISA Kit be used to detect Cas9 residues from other companies?

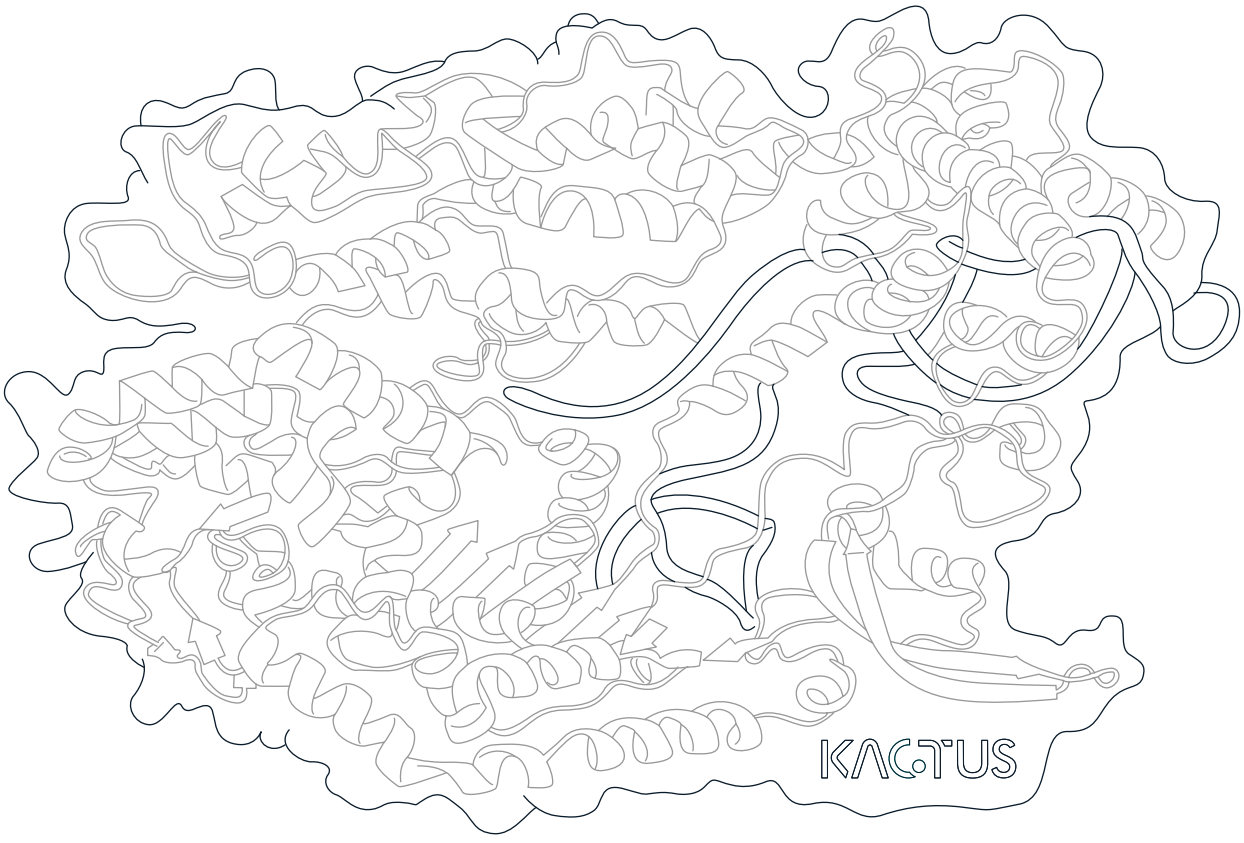
Our Cas9 (CRISPR Associated Protein 9) ELISA Kit has been tested against multiple leading Cas9 enzyme vendors and was able to accurately detect and quantity all Cas9 enzymes tested.

Ordering Information

Catalog #	Product Description	Available Sizes
CAS-EE109	CRISPR Cas9 Protein (Research-Grade)	100µg / 1mg
GMP-CAS-EE109	CRISPR Cas9 Protein (GMP-Grade)	3mg
CAS-MM00B	Cas9 ELISA Kit	96T

Request a Quote or More Information

Please contact support@kactusbio.us to request a quote or additional information for one of our gene editing enzymes or reagents. One of our team representatives would be happy to speak with you!



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