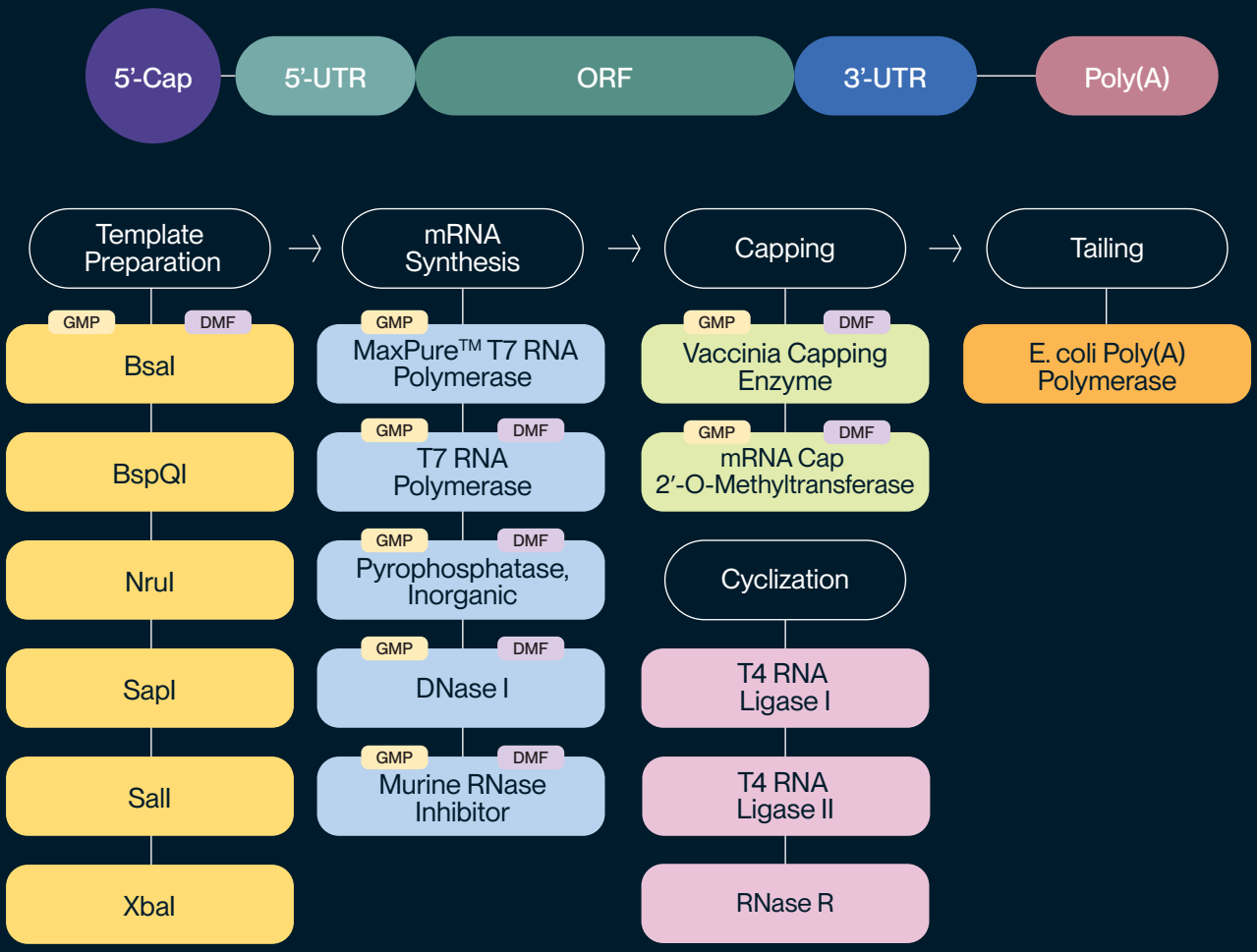
KACTUS

GMP-Grade mRNA Production Enzymes



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United States

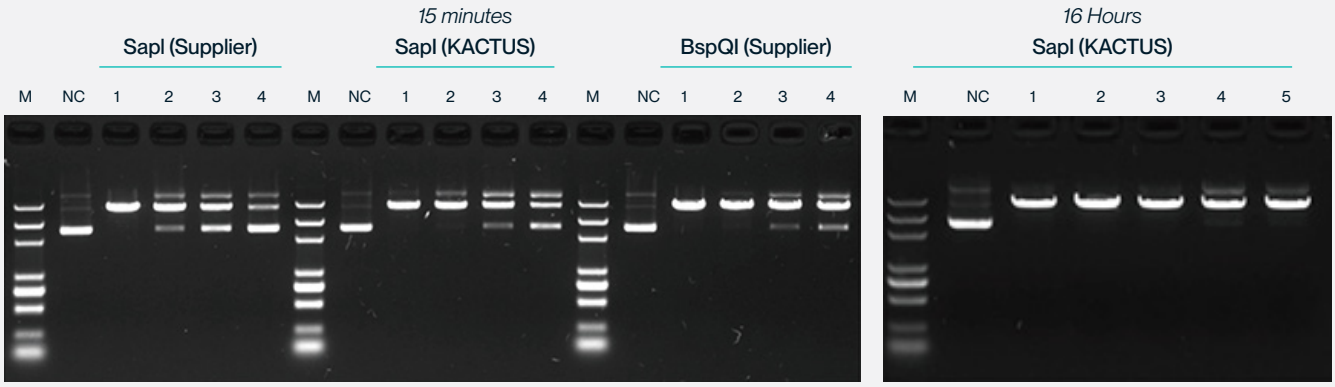
Restriction Endonucleases

Linearized templates are acquired through the enzymatic digestion of plasmids using restriction endonucleases. This process ensures the creation of DNA fragments with either blunt ends or a 5' overhang. Specifically, the utilization of Type IIS restriction endonucleases is recommended. These enzymes cleave DNA sequences outsidetheir recognition sites, thus producing DNA fragments with a 5' overhang post-digestion. KACTUS offers a series of activity-tested restriction endonucleases for plasmid linearization.

SapI

#SAP-SE101

SapI is a type IIS restriction endonuclease with the cut site GCTCTTC(1/4). It recognizes non-palindromic DNA sequences and cuts outside the recognition sequence to produce sticky ends. Additionally, we have engineered SapI into our SapI V2.0, which can more efficiently cut challenging plasmids.



(A) The cutting efficiency of SapI is superior to that of a well-known supplier, and is comparable to BspQI. (A) In a 50μL standard reaction system, 1μg of plasmid and 1μL of SapI/BspQI were added (with a negative control well and wells 1-4 having a two-fold gradient dilution). The plasmid was digested at 37°C/50°C for 15 minutes. (B) SapI at 37°C showed no star activity after 16 hours of digestion. In a 50μL standard reaction system with 1μg plasmid and 1μL SapI added (with a negative control well and wells 1-5 having a two-fold gradient dilution)

SapI V2.0

#SAP-SE102

KACTUS has engineered SapI V2.0 to perform well in cutting challenging plasmids.

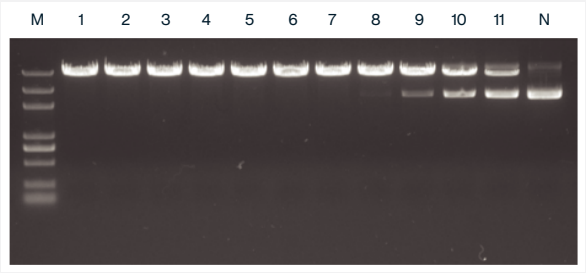
For difficult-to-cut plasmids, the enzymatic cutting performance of SapI V2.0 is superior to that of other suppliers. In a 50μL reaction system, 1μg of plasmid and 1μL of SapI were added (with a two-fold gradient dilution and a negative control well). The plasmids were digested at 37°C for 6 hours

BsaI, GMP-Grade

#GMP-BSA-EE101

BsaI is a type IIS restriction endonuclease with the cut site GGTCTC(1/5). It offers high specificity, effectively avoiding nonspecific digestion. Our BsaI is manufactured GMP-Grade and has been submitted to the FDA Drug Master Files (#037503).

(Right) Activity of BsaI digestion. In a 20μL system, 1μg of plasmid and 1μL of BsaI were added (well 1 contains the undiluted enzyme, and wells 1-11 have a twofold gradient dilution). The mixture was digested for 30 minutes.



BspQI

#BSP-BE101

BspQI is a type IIS restriction endonuclease that can recognize non-palindromic DNA sequences and cuts outside the recognition sequence to produce sticky ends. The cut site is GCTCTTC(1/4).

BspQI has good digestion activity, with no star activity observed after 16 hours of digestion. In a 50μL standard reaction system, 1μg of plasmid and 1μL of BspQI were added (with a negative control well and wells 1-5/7 having a two-fold gradient dilution). The plasmid was digested at 50°C for 15 minutes (left, middle) or 16 hours (right).

XbaI

#XBA-EE101

The XbaI restriction endonuclease can be used for preparing linear templates, with its cutting site at T/CTAGA.

The cutting performance of KACTUS' XbaI is superior to that of a leading supplier. In a 50μL reaction system, 1μg of plasmid and varying amounts of XbaI (as shown in the figure) were added, and the mixture was digested for 30 minutes.

MaxPure™ T7 RNA Polymerase

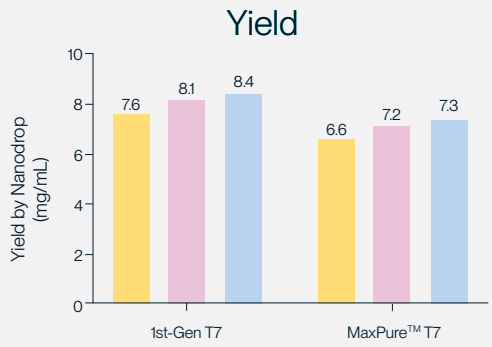
Engineered T7 RNA Polymerase for IVT with Low dsRNA

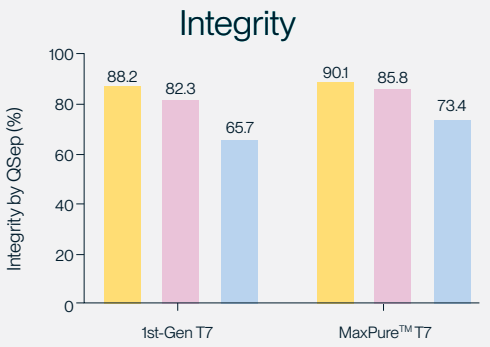
T7 RNA Polymerase is the key enzyme for in vitro transcription (IVT) of mRNA. T7 RNA Polymerase is a DNA-dependent polymerase that efficiently incorporates standard or modified nucleoside triphosphates (NTPs) into RNA transcripts. T7 RNA Polymerase initiates transcription at the G* in the classic T7 promoter sequence: taatacgactcactataG*GG, and is highly specific to its promoter sequence.

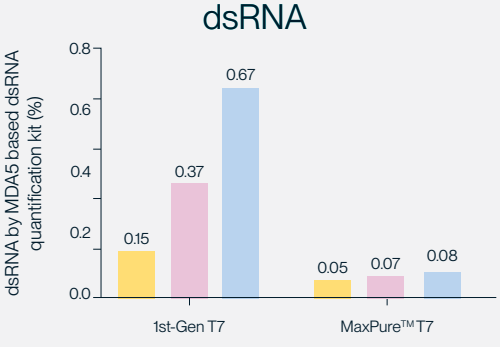
KACTUS has engineered two generations of T7 RNA Polymerase, optimized for yield, purity, and reduced dsRNA. MaxPure™ T7 RNA Polymerase is our second-generation mutant developed by modifying key sites of the first-generation T7 RNA Polymerase. Compared to the first-generation T7 RNA Polymerase, MaxPure™ T7 RNA Polymerase more effectively reduces the content of dsRNA byproducts. Moreover, it can ensure a high capping rate without affecting the yield and purity, even when the concentration of cap analogs in the co-transcriptional capping system is low.

Parameter	First Generation T7 RNA Polymerase (#GMP-T7P-EE101)	Second Generation MaxPure™ T7 RNA Polymerase (#GMP-T7P-EE1MP)
Yield & Purity	High	High
dsRNA in Transcripts	Moderately reduced	Significantly reduced
Optimization of *E. coli* Expression System	✓	✓
Optimization of Purification Process	✓	✓
Optimization of Reaction Buffer	✓	✓
Capping Efficiency	High efficiency at standard cap analog concentrations	High efficiency at extreme cap analog concentrations (as low as 1mM)
Manufactured According to cGMP Guidelines	✓	✓
Registered with FDA Drug Master Files	#037660	Submission in progress

Effectively reduce production of dsRNA byproducts







mRNA, 2160nt, co-capping IVT, N1-Ψ

mRNA, 4300nt, co-capping IVT, N1-Ψ

saRNA, 9700nt, co-capping IVT, UTP

The first generation T7 and second generation MaxPure™ T7 were used for co-transcriptional capping on different templates to synthesize mRNA and saRNA of different lengths. After purification by lithium chloride precipitation, the yield, integrity and dsRNA content were measured. The results show that MaxPure™ T7 RNA Polymerase does not affect the yield and integrity of RNA for different templates, and can effectively reduce the production of dsRNA.

Achieve highly efficient mRNA capping, even in extreme capping analog concentrations

Cap Analog Concenteration	MaxPure™ T7 vs 1st-Gen T7											
	1mM		2mM		3mM		4mM		5mM		6mM	
	1st-Gen T7	MaxPure™ T7	1st-Gen T7	MaxPure™ T7	1st-Gen T7	MaxPure™ T7	1st-Gen T7	MaxPure™ T7	1st-Gen T7	MaxPure™ T7	1st-Gen T7	MaxPure™ T7
Yield by Nanodrop (mg/mL)	5.36	5.65	5.43	6.18	6.78	5.92	6.78	6.72	6.72	6.9	7.2	7.02
Integrity by QSep (%)	83.4	82.3	83.7	81.3	82.8	87.2	85.1	84.6	84.9	87.3	86.8	89.2
Capping efficiency by LC-MS (%)	**88.24**	**97.94**	**92.73**	**97.75**	97.83	98.44	97.52	99.39	97.95	99.67	97.94	99.82

Co-transcriptional capping (each NTP 7.5 mM) was performed using 1st-gen T7 and MaxPure™ T7 to determine whether the capping efficiency was affected at different cap analog concentrations (1 ~ 6 mM). The results show that MaxPure™ T7 RNA Polymerase does not affect yield and integrity at the low cap analog concentration (1 mM), and and the capping efficiency of our MaxPure™ T7 was superior to that of our first-generation T7.

Product List: T7 RNA Polymerase

Catalog #	Product Description	Sizes
GMP-T7P-EE101	T7 RNA Polymerase, GMP-Grade	50kU / 1MU
T7P-EE1MP	MaxPure™ T7 RNA Polymerase*, Research-Grade	20KU / 100KU / 200KU
GMP-T7P-EE1MP	MaxPure™ T7 RNA Polymerase*, GMP-Grade	200KU / 2MU

*MaxPure™ T7 RNA Polymerase is the commercial name for Premium T7 RNA Polymerase.

Quality Control Specifications

MaxPure™ T7 RNA Polymerase

#GMP-T7P-EE1MP

Parameter	Acceptance Criteria
Identity	Corresponding to reference standards
Activity	200.0-320.0 kU/mL
pH Value	7.9±0.5
Purity	≥ 95.0%
Bacterial Endotoxin	≤ 10.0 EU/mL
Residual DNase	Negative
Residual RNase	Negative
Residual Protease	Negative
Nickel Salt Residue	≤ 10.0 ppm
Heavy Metal Residue	≤ 10.0 ppm
Bioburden	≤ 1 CFU/10mL
Concentration	3.2 mg/mL±20%

Product Specifications

MaxPure™ T7 RNA Polymerase

#T7P-EE1MP or #GMP-T7P-EE1MP

Parameter	Specification
Express System	*E. coli*
Concentration	200U/μL
Unit Definition	One unit is defined as the amount of enzyme required to incorporate 1 nmol ATP into acid-insoluble material in a total reaction volume of 50μL in 1 hour at 37°C.
Storage Buffer	50 mM Tris-HCl, 100 mM NaCl, 2mM DTT, 1 mM EDTA, 50% Glycerol, 0.1% Triton X-100, pH 7.9

Request a Quote or More Information

Please contact **support@kactusbio.us** to request a quote or additional information for our T7 RNA Polymerase or other mRNA enzymes. One of our team representatives would be happy to speak with you!

MDA5 dsRNA Quick-Quantification Kit

#MD-DS00B

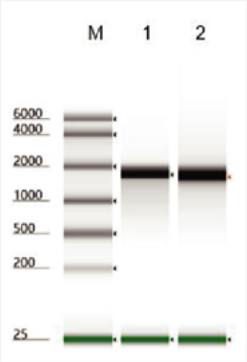
KACTUS has developed a spectrophotometric MDA5-based dsRNA quantification kit to analyze dsRNA byproducts in mRNA samples. The MDA5 kit offers fast, reliable dsRNA quantification, with a linear range of 0.078-5ng/μL and sensitivity up to 0.078ng/μL.

In Vitro Transcription Enzymes

Pyrophosphatase, Inorganic, GMP-Grade

#GMP-PYR-YE101

Hydrolyzes inorganic pyrophosphate generated during in vitro transcription reactions to increase RNA yield

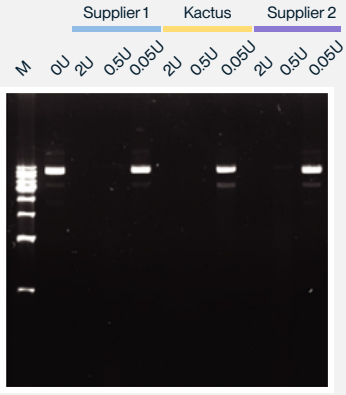


Inorganic pyrophosphatase was added to a 20μL in vitro transcription system. Well 1 did not have it added, while well 2 had it added. Inorganic pyrophosphatase effectively increases mRNA yield.

DNase I (RNase-free), GMP-Grade

#GMP-DNI-EE001

Digests single - & double - Stranded DNA for mRNA purification



The effectiveness of DNase I in digesting DNA is demonstrated. In a 20μL system, 1μg of DNA and varying amounts of DNase I were added. Compared with suppliers 1 and 2, KACTUS' DNase I shows superior digestion performance.

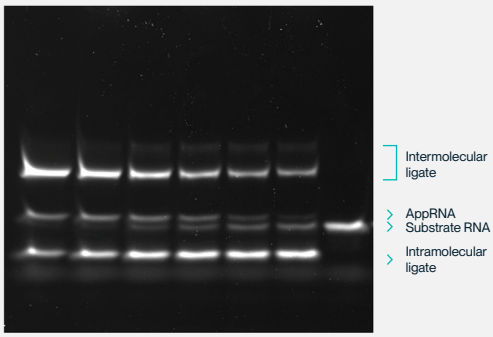
T4 RNA Ligases

Linear mRNA obtained through in vitro transcription (IVT) can be further cyclized to produce circular RNA (circRNA). Cyclization methods include Group I intron self-splicing, Group II intron self-splicing, and T4 RNA Ligase, among others. KACTUS provides T4 RNA Ligase 1 and 2 and RNase R for the in vitro cyclization and purification of circRNA, supporting the industrial development of circRNA therapies.

T4 RNA Ligase I

#TRL-BE101

Catalyzes the 5' phosphate group of the donor and the 3' hydroxyl group of the ligand to form a 3' → 5' phosphodiester bond

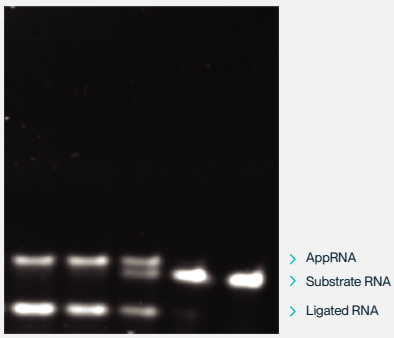


Ligation of a 15mer RNA substrate using T4 RNA ligase I, followed by Urea page gel analysis.

T4 RNA Ligase II

#TRL-BE103

ATP-dependent RNA ligase that catalyzes the inter- and intramolecular RNA strand joining activity via phosphodiester bond formation.



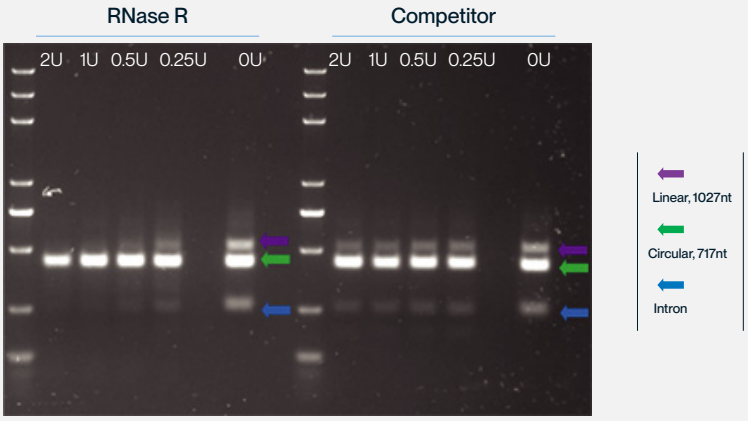
Ligation of a 22mer RNA substrate using T4 RNA ligase II, followed by Urea page gel analysis.

Ribonucleases

RNase R: Engineered for Improved Specificity for Linear RNA

#RNR-EE001

Digests linear RNA without affecting circular RNA.



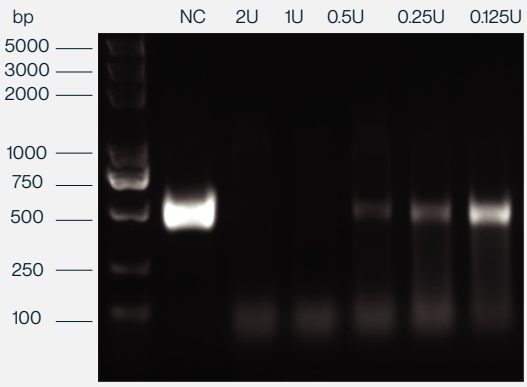
Circular RNA 1

The effects of RNase R digestion on linear RNA and circRNA. In a 20μL system, 1μg of circular RNA was loaded with varying amounts (shown in figure) of RNase R. The results demonstrate that RNase R can effectively digest linear RNA but it has no effect on circRNA.

RNase III

#RNI-EE601

Double-stranded RNA (dsRNA) -specific endoribonuclease that can cleave dsRNA into 18-25 bp interfering RNAs (siRNA) with 2 nucleotide 3' overhangs, 5' phosphate and 3' hydroxyl.

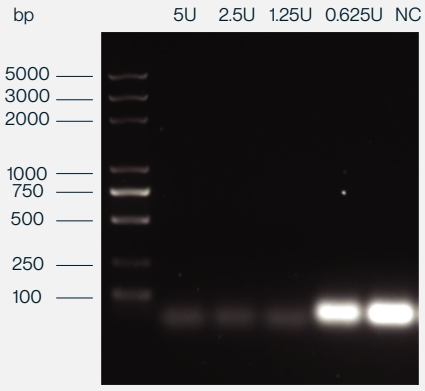


Digestion of 1μg 500bp dsRNA with RNase III, followed by Agarose gel analysis.

RNase H

#RNH-EE101

Endoribonuclease that can specifically hydrolyze the phosphodiester bonds of RNA and degrades the RNA strand in the RNA-DNA hybrid



Digestion of a RNA-DNA hybrid substrate with RNase H, followed by Agarose gel analysis.

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. Our QMS and GMP facilities have passed audits and been recognized by various pharmaceutical companies. We have successfully assisted multiple clients in completing Investigational New Drug (IND) applications.

- Strict management of cell strains, segmented into Master Cell Banks (MCB) and Working Cell Banks (WCB), overseen with multi-tiered personnel oversight and layered security protocols
- Animal-origin free
- Thorough inspection and quality approval of all raw materials, auxiliary ingredients, and packaging materials utilized in the production process prior to release
- Stringent control of key parameters ensures consistent batch-to-batch quality during production
- Systematic validation of analytical methods to ensure the validity and reproducibility of results
- Comprehensive quality control and release testing conducted on in-process samples, raw materials, intermediates, and finished products
- Continuous monitoring of batch-to-batch variances to ensure consistency across batches
- Validation of product stability, including influencing factor testing, accelerated stability studies, and long-term stability studies

GMP Manufacturing Facility

500L Dosing and Cleaning In Place (CIP) System

1000L Fermentation Tank

Isolator

Chromatography System

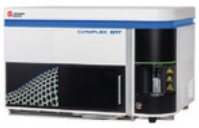
Regulatory Documentation Support

KACTUS can provide the corresponding basic product documents listed below for preclinical research. In addition, if your biopharmaceutical product will be entering the application stage, we will provide additional supporting documents as needed to assist you with your application. Our documentation package is cutomizable so please contact a represenative to discuss your specific regulatory standards.

Basic Product Documentation
Certificate of Analysis
TSE/BSE Statement
Certificate of Origin
Drug Master File (if applicable)
Safety Data Sheet

Additional Supporting Documentation
Bacterial strain identification and passage stability test report
Process Validation Report
Analycial Method Validation Report
Batch Production Records
Batch Inspection Records
Product Stability Report

Comprehensive Suite of Analytical Equipment



CytoFLEX SRT
Flow Sorter

Biacore™ T200

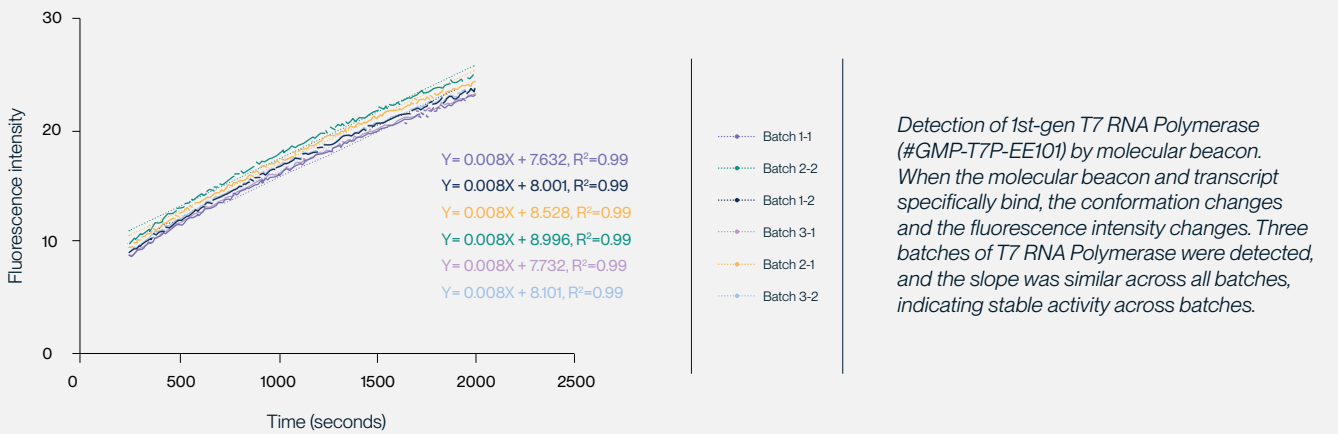
6545XT Q-TOF (time-of-flight)
Mass Spectrometer

Multifunctional
Microplate Reader

Capillary
Electrophoresis Instrument

1260 High-Performance
Liquid Chromatograph

Batch Consistency



Detection of 1st-gen T7 RNA Polymerase (#GMP-T7P-EE101) by molecular beacon. When the molecular beacon and transcript specifically bind, the conformation changes and the fluorescence intensity changes. Three batches of T7 RNA Polymerase were detected, and the slope was similar across all batches, indicating stable activity across batches.

Frequently Asked Questions

IVT Reaction

What products do you offer for an IVT reaction?

We have Restriction Enzymes (BsaI, BspQI, NruI, SalI, SapI, XbaI) for plasmid linearization and IVT enzymes including T7 RNA Polymerase, Murine RNase Inhibitor, Pyrophosphatase, DNase 1, and capping system that includes Vaccinia capping enzyme and mRNA Cap 2'o-Methyltransferase.

What capping system is suitable for your IVT reaction?

KACTUS T7 RNA polymerases support both a cotranscriptional system using cap analog and a vaccinia capping system. We have two GMP-grade capping enzymes: mRNA Cap 2'o-Methyltransferase and Vaccinia capping enzyme.

Do you provide a recommended protocol for using your IVT reagents?

Yes, this can be found on the product page where there is an option to download the datasheet which contains the protocol.

GMP-Grade Products

What GMP grade products for IVT does KACTUS have?

KACTUS has GMP-grade T7 RNA Polymerases, Murine RNase Inhibitor, Pyrophosphatase, DNase I.

There is an enzyme I want that is not GMP-grade. Can you make this GMP grade?

Yes, we can scale up a GMP-grade enzyme based on the enzyme and quantity needed. Please contact us at support@kactusbio.us.

What is the difference between GMP-ready and GMP-grade?

GMP-GRADE: Manufactured according to cGMP guidelines.

GMP-READY: Industrial-grade manufacturing transferrable to GMP-level manufacturing.

Frequently Asked Questions

T7 RNA Polymerase

Do KACTUS T7 RNA Polymerases support different forms of RNA?

Yes! KACTUS T7 RNA Polymerases can be used for linear mRNA, circular RNA, self amplifying RNA, and more.

Are KACTUS T7 RNA Polymerases engineered?

Yes, both our 1st-gen T7 RNA Polymerase (#GMP-T7P-EE101) and second-generation MaxPure™ T7 RNA Polymerase (#GMP-T7P-EE1MP) are engineered. See page 4 and 5 for additional product information. We also have additional variants that we've established in our engineering process that have high yield and less double stranded RNA. Currently we have 4 variants that have different applications such as circRNA or long RNAs. Please contact us to learn more about getting a sample to test.

Does KACTUS provide a buffer with the T7 RNA Polymerase?

Yes, the buffer provided with both our T7 RNA Polymerases was highly optimized and the components are confidential.

What is the concentration of the T7 RNA Polymerase?

The concentration of the 1st-gen T7 RNA Polymerase is 50U/uL and the MaxPure™ T7 RNA Polymerase is 200U/µL.

How long can the IVT enzymes be stored?

The IVT enzymes should all be stored in a -20C freezer for 24 months.

Product List

Application	Catalog #	Product Description	Grade
Restriction Endonuclease	GMP-BSA-EE101	BsaI	GMP-Grade
Restriction Endonuclease	BSP-BE101	BspQI	GMP-Ready
Restriction Endonuclease	NRU-RE101	NruI	GMP-Ready
Restriction Endonuclease	SAL-SE101	SalI	GMP-Ready
Restriction Endonuclease	SAP-SE101	SapI	GMP-Ready
Restriction Endonuclease	SAP-SE102	SapI V2.0	GMP-Ready
Restriction Endonuclease	XBA-EE101	XbaI	GMP-Ready
In Vitro Transcription	GMP-DNI-EE001	DNase I	GMP-Grade
In Vitro Transcription	GMP-RNI-ME101	Murine RNase Inhibitor	GMP-Grade
In Vitro Transcription	GMP-PYR-YE101	Pyrophosphatase, Inorganic	GMP-Grade
In Vitro Transcription	GMP-T7P-EE101	T7 RNA Polymerase	GMP-Grade
In Vitro Transcription	GMP-T7P-EE1MP	MaxPure™ T7 RNA Polymerase*	GMP-Grade
In Vitro Transcription	T7P-EE1MP	MaxPure™ T7 RNA Polymerase*	Research-Grade
Capping	GMP-MEH-VE101	mRNA Cap 2'-O-Methyltransferase	GMP-Grade
Capping	GMP-VCS-VE101	Vaccinia Capping Enzyme	GMP-Grade
Ribonuclease	RNR-EE001	RNase R	GMP-Ready
Ribonuclease	RNI-EE601	RNase III	GMP-Ready
Ribonuclease	RNH-EE101	RNase H	GMP-Ready
Circular RNA	TRL-BE101	T4 RNA Ligase I	GMP-Ready
Circular RNA	TRL-BE103	T4 RNA Ligase II	GMP-Ready
Tailing	PLA-EE101	E.coli Poly (A) Polymerase	GMP-Ready
dsRNA Quantification	MD-DS00B	MDA5 dsRNA Quick-Quantification Kit	Research-Grade

GMP-Ready indicates the product is manufactured industrial grade and can be transferred to our cGMP manufacturing facility in accordance with your needs. For more information, please contact a representative at support@kactusbio.us

*MaxPure™ T7 RNA Polymerase is the commercial name for Premium T7 RNA Polymerase.

Learn more