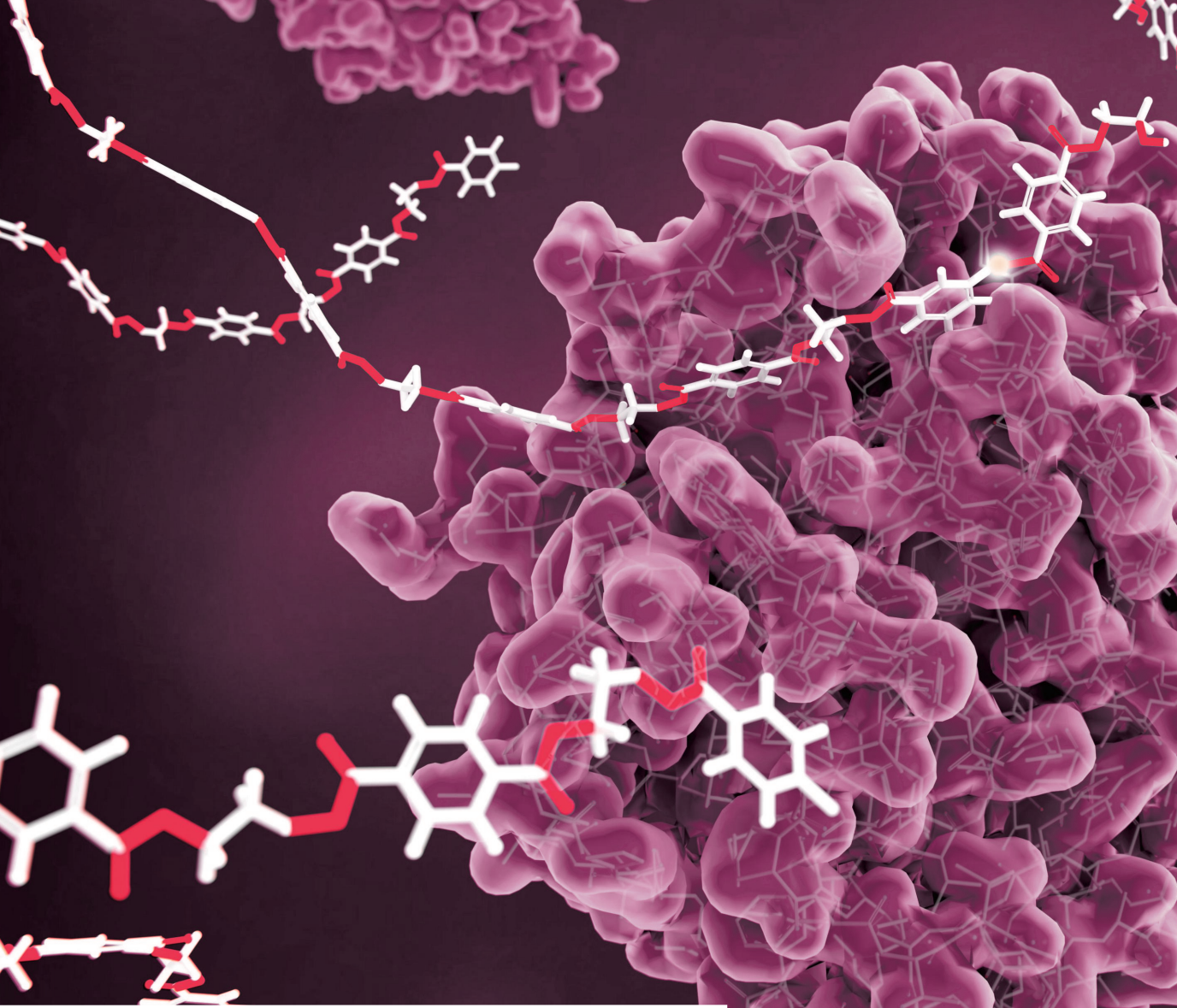

Enzymes Program for

mRNA VACCINE

KRISHGEN BioSystems

OUR REAGENTS. YOUR RESEARCH.

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01 ABOUT US

Established in 2003, KRISHGEN BIOSYSTEMS is a leading supplier of reagents and assay kits to the life sciences industry. We have evolved to offer our own manufactured products for life sciences, biopharmaceutical, vaccine and hospitals and laboratories for our in vitro diagnostic kits. Our R&D is at Worli, Mumbai and Bhiwandi, Thane with production centre in Bhiwandi, Thane.

We are also working on setting up our state of the art GMP and non GMP suites for our products in India.

Based on its technical platforms for immunology and cell culture, KRISHGEN has developed range of immunoassays including ELISAs, enzymatic assays and biochemical kits. Our R&D has been succesful in also developing through bacterial expression, enzymes required for the biopharmaceuticals and vaccine industry.

KRISHGEN has been instrumental in developing assays specially on the ELISA platform since 2008 when the first commercial kits were launched in India. Since then over the years, we have increased the portfolio of products and taken then to international markets including US. Europe, and rest of Asia.

Post Covid-19, we have started our work on protein expression and harnessing our skills working with leading cell culture and protein expression companies. Our lab level pilot bacterial cell culture and mammalian cell culture facility is operational.

We are proud to now offer these new product coming from there. We are also in the midst to set up our new facility (GMP and non GMP suites) for these products and hope to be operational by 2024 first quarter.

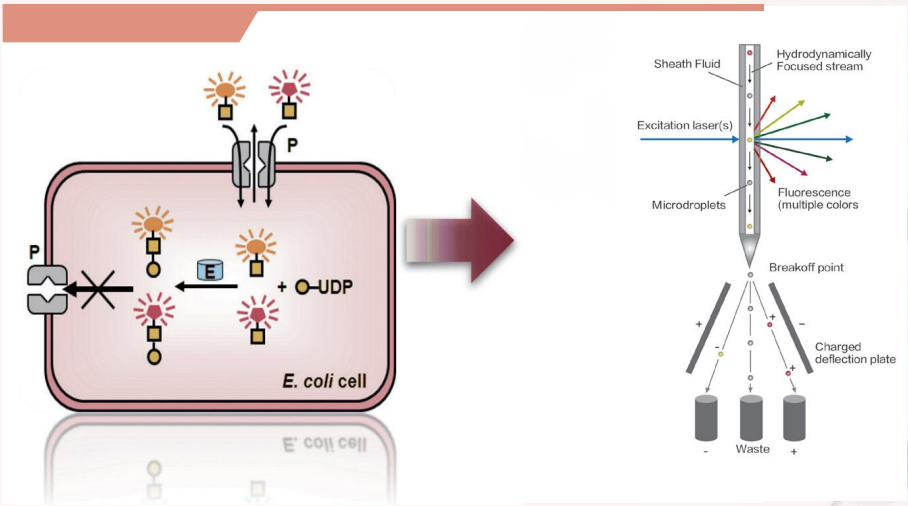
Our Reagents - Your Research

Delivering Science with Quality and Affordable Solutions

Technology Platform

Scalable Cell Culture from Lab to Production

The intrinsic properties of natural enzymes have confined their appli-cations in the pharmaceutical industry. Directed evolution of enzymes – the pioneering technology first introduced by the 2018 Nobel Prize in Chemistry winner Dr. Frances H. Arnold. She introduces the concept of evolution into enzyme molecular modification, making it possible for hundreds of millions of natural enzymes uphold for the good of human beings.



Krishgen has worked with enabling technologies using its skill sets and aquiring new ones on the way, to offer quality reagents at affordable costs with consistent quality to help lower your costs of research and production.

Based on new age disposable cell culture to achieve the highest protein and enzyme expression coupled with innovative purification techniques to achieve increased yields and low costs.

We continue to focus on our cell culture skills to ensure with add more product(s) to our portfolio over the coming years.

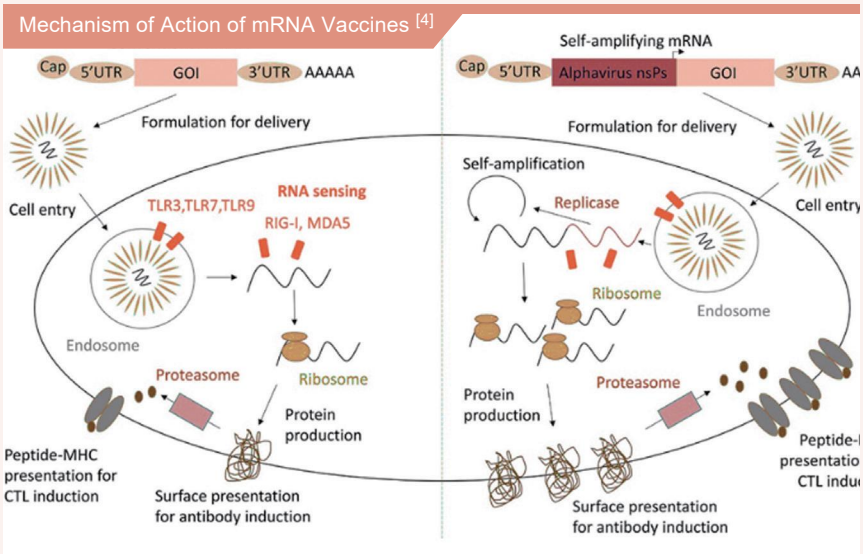
higher working volumes and improved aeration increase efficiency to increased cell expansion - we call it more art and not science to cell culturing

02 Enzymes Program for mRNA Vaccine

mRNA Vaccine

mRNA Vaccine is a new vaccine which can induce adaptive immune response by introducing pathogenic mRNA or cancer cell mRNA, thereby triggering adaptive immune response to achieve immune effect. The core component of mRNA vaccine is the mRNA molecule encoding antigen [1]. These mRNA molecules are coated by special carriers such as solid lipid nanoparticles, which function to protect these relatively fragile mRNA molecules and to assist them in entering cells [2] [3].

Mechanism of Action of mRNA Vaccines [4]



The study of mRNA vaccines will provide a new means to save the mankind from pathogenic microorganism infection and improve the cure rate of tumors. The advantages of mRNA vaccines over conventional vaccines are as follows:

1. mRNA vaccines simultaneously induce cellular immunity and humoral immunity - high immunization effect
2. mRNA vaccines do not enter cell nucleus - no risk of exogenous gene integration

3 mRNA vaccines encode a large number of epitopes - suitable for vaccines encoding tumor-associated epitopes

4 mRNA vaccines do not require processes such as cell culture - low cost and high produc-tion efficiency

Nevertheless, little is known of the long-term side effects of mRNA vaccines, which also have autoimmunity risk [5].

With the wide clinical application of mRNA vaccines, the technology roadmap for stable and efficient synthesis of mRNA has also gradually matured, which poses higher quality requirements for enzymes and other relevant raw materials for synthesizing mRNA.

Product List

Item No.	Name	Product Overview
KNB9001	T7 RNA Polymerase	Efficient synthesis of mRNA; recombinant expression in E.*coli*.
KNB9002	Vaccinia Capping Enzyme	Adding the 7-methylguanosine cap (Cap0) to the 5' terminus of RNA to improve mRNA stability and translation efficiency and reduce the immunoge-nicity of mRNA; derived from recombinant expression of vaccinia virus in E.*coli*.
KNB9003	mRNA Cap-2'-O-Methyltransferase	Specifically transferring the methyl of methylation donator SAM to the Cap0 structure in RNA to form the Cap1 structure and improve mRNA translation efficiency; derived from recombinant expression of vaccinia virus in E.*coli*.
KNB9004	Poly(A) Polymerase	With ATP as the substrate, adding adenyl acid to the 3'-hydroxyl terminus of RNA to form the PolyA tail structure, which can enhance mRNA stability and translation efficiency, and can be used as the target for Oligo dT purification to purify RNA; recombinant expression of Poly (A) Polymerase in E.*coli*.
KNB9005	RNase inhibitor (Recombinant)	Inhibiting the activity of RNase A, RNase B and RNase C through specific binding; protecting RNA from degradation; recombinant expression of murine RNase inhibitor in E.*coli*.
KNB9006	DNase I	Shearing the endonuclease of single-stranded or double-stranded DNA, to efficiently remove DNA template; recombinant expression in E.*coli*.
KNB9007	RNase III	Acting on double-stranded RNA (dsRNA), the product is 18-25bp siRNAs with 5'-PO4, 3' -OH, 3' -end projecting two nucleotides, suitable for RNAi in mammalian cells; recombinant expression in E.*coli*.
KNB9008	T4 RNA ligase	ATP-dependent ligase, catalyzing 5'-phosphoric acid and 3'-OH to form phosphodiester bond; capable of RNA cyclization and 3' labelling; recombinant expression in E.*coli*.
KNB9009	Pyrophosphatase Inorganic (yeast)	Hydrolyze the inorganic pyrophosphate produced in the nucleic acid ampli-fication experiment, to avoid its inhibitory effect on the reaction system, and increase the yield of reaction products including IVT and PCR; recombinant expression in E.*coli*.
KNB9010	Alkaline Phosphatase	Non-specific catalysis of 5 'and 3' ends of DNA and RNA, to further reduce the immunogenicity of mRNA; recombinant expression in E.*coli*.
KNB9011	EcoR I	High-efficiency linearization of the gene of interest; recombinant expression in E.*coli*.

T7 RNA Polymerase

Product Introduction

This product is the phage T7 RNA Polymerase derived from recombinant expression in E. coli. It can specifically recognize the T7 promoter sequence, and synthesize RNA complementary to downstream single-stranded DNA of the promoter using the single or double stranded DNA containing the sequence of T7 promoter as the template and NTP as the substrate.

Product Composition

Item No.	Composition	Storage Temperature (℃)	Product ID / Specification	
			KNB9001S (5KU)	KNB9001L (50KU)
KNB9001-I	T7 RNA Polymerase (50 U/ul)	-20	100 ul	1000 ul
KNB9001-II	Buffer	-20	400 ml	4000 ml

1X Buffer contains: 40 mM Tris HCl (pH7.9), 6mM $MgCl_2$, 1mM DTT and 2mM spermidine, 10mM NaCl.

Product Properties

Optimal Reaction Temperature: 37℃

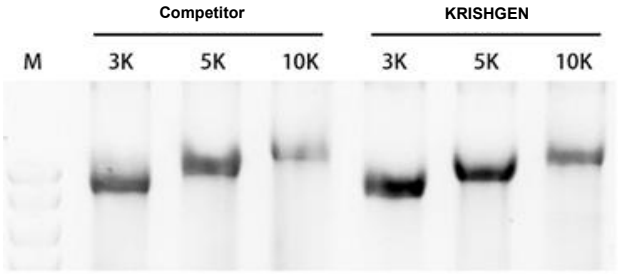
Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to incorporate 1 nmol of [^{3}H] GMP into acid-insoluble precipitate within 1h under the conditions of 37℃ and pH8.0.

Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100 pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

Product Features

Excellent RNA output rate; homogeneous transcript length; transcript length up to 10K.



Product Information

Item No.	Name	Specification
KNB9001	T7 RNA Polymerase	5KU, 50KU

Vaccinia Capping Enzyme

Product Introduction

This product is the Vaccinia Capping Enzyme recombinantly expressed in E. coli. Vaccinia Capping Enzyme can add the 7-methylguanosine cap (Cap0) to the 5' terminus of mRNA. This structure can improve the stability of mRNA, and is indispensable to subsequent transport and translation.

Product Composition

Item No.	Composition	Storage Temperature (℃)	Product ID / Specification	
			KNB9002S (500 U)	KNB9002L (2000 U)
KNB9002-I	Vaccinia Capping Enzyme (10 U/ul)	-20	50 ul	200 ul
KNB9002-II	Capping Buffer	-20	100 ul	1000 ul
KNB9002-III	S-adENosylmethionine (SAM 32mM)	-20	100 ul	1000 ul
KNB9002-IV	GTP	-20	50 ul	500 ul

10X Capping Buffer contains: 0.5M Tris-HCl (pH 8.0), 50mM KCl, 10mM $MgCl_2$, 10mM DTT.

Product Properties

Optimal Reaction Temperature: 37°C

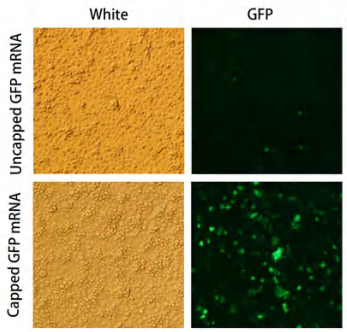
Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to incorporate 10 pmol of (α^{32}P) GTP into 80nt transcript within 1h at 37°C .

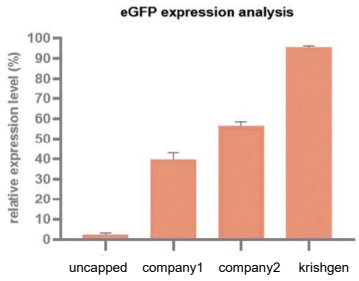
Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100 pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

Product Features

Capping rate as high as 95%; the 5'-Cap structure effectively promting the in vivo expression of mRNA.





Product Information

Item No.	Name	Specification
KNB9002	Vaccinia Capping Enzyme	500 U, 2000 U

mRNA Cap-2'-O-Methyltransferase

Product Introduction

This product is the mRNA Cap-2'-O-Methyltransferase of vaccinia virus recombinantly expressed in E.*coli.* With the RNA of 7-methylguanosine cap (Cap0) as the substrate, it specifically transfers the methyl of methylation donator SAM to the Cap0 structure in RNA to form the Cap1 structure, which can enhance the translation efficiency of mRNA and avoid the innate immune response in certain cells.

Product Composition

Item No.	Composition	Storage Temperature (℃)	Product ID / Specification	
			KNB9003S (2.5 KU)	KNB9003L (10 KU)
KNB9003-I	mRNA Cap-2'-O-Methyltransferase (50 U/ul)	-20	50 ul	200 ul
KNB9003-II	Reaction Buffer	-20	100 ul	1000 ul
KNB9003-III	S-adENosylmethionine (SAM 32mM)	-20	100 ul	1000 ul

10X Reaction Buffer contains: 0.5M Tris-HCl (pH8.0), 50mM KCl, 10mM $MgCl_2$ and 10mM DTT.

Product Properties

Optimal reaction temperature: 37°C

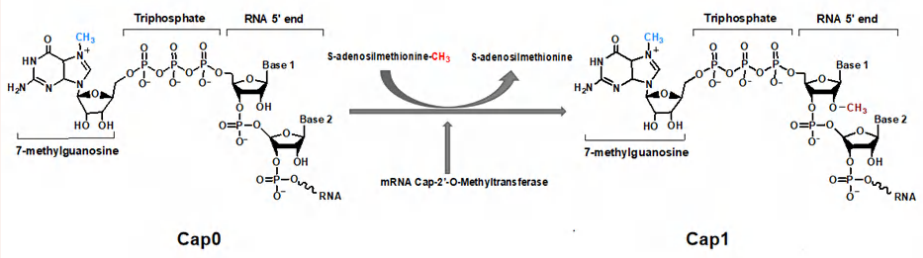
Definition of active unit: 1 active unit is defined as the amount of enzyme needed to incorporate 10 pmol of (α^{32}P)GTP into 80nt transcript within 1hr at 37°C.

Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100 pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

Product Features

In vitro capping can avoid in vivo immune response.



Product Information

Item No.	Name	Specification
KNB9003	mRNA Cap-2'-O-Methyltransferase	2.5 KU, 10 KU

Poly (A)Polymerase

Product Introduction

This product is the Poly(A) Polymerase recombinantly expressed in E.*coli*. It catalyzes with ATP as the substrate, and adds adenyl acid to the 3'-hydroxyl terminus of RNA to form the PolyA tail structure in the form of AMP.

Product Composition

Item No.	Composition	Storage Temperature (℃)	Product ID / Specification	
			KNB9004S (100U)	KNB9004L (1000U)
KNB9004-I	Poly(A) Polymerase (5 U/ul)	-20	50 ul	200 ul
KNB9004-II	Reaction Buffer	-20	1.5 ml	15 ml
KNB9004-III	AdENosine-5'-Triphosphate (ATP 10 mM)	-20	200 ul	2000 ul

1X Reaction Buffer contains 0.5M Tris-HCl (pH8.0), 50mM KCl and 10mM $MgCl_2$.

Product Properties

Optimal Reaction Temperature: 37℃

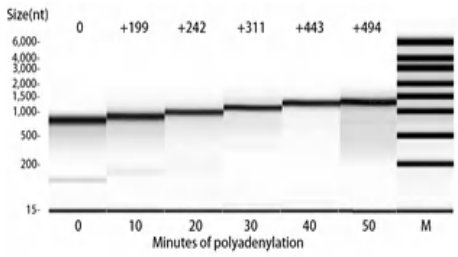
Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to incorporate 1 nmol of AMP into RNA within 10 min at 37℃ in a 20 ul reaction system.

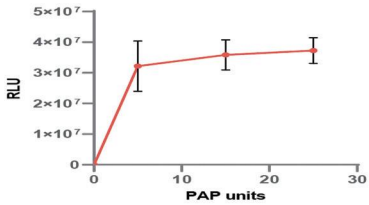
Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100 pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

Product Features

Efficient mRNA capping: adding 200nt polyA tail to 10 ug of RNA in 10 min (5 U enzyme for capping). Homogenous tailing length: controlling polyA tail length by adjusting reaction time or enzyme dosage.





Product Information

Item No.	Name	Specification
KNB9004	Poly(A) Polymerase	100 U, 1000 U

RNase Inhibitor (Recombinant)

Product Introduction

This product is the murine RNase inhibitor recombinantly expressed in E.*coli*. It binds to RNase to form a complex, thereby inhibiting RNase activity and protecting target RNA from degradation.

Product Composition

Item No.	Composition	Storage Temperature (°C)	Product ID/Specification	
			KNB9005S (2.5 KU)	KNB9005L (10 KU)
KNB9005-I	RNase Inhibitor (Recombinant) (40 U/ul)	-20	62.5 ul	250 ul

Product Properties

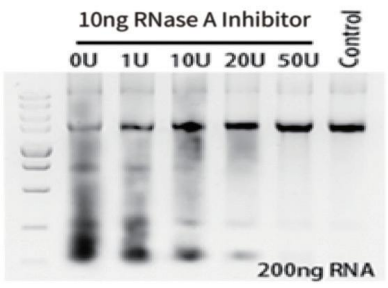
Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to inhibit 50% of RNase A activity (RNase A activity is determined by inhibiting its hydrolysis of cytidine 2' and 3'-cyclic monophosphate).

Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100 pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

Product Features

Efficient inhibition of the activity of RNase A, RNase B and RNase C, no nuclease contamination, no residual microbial-derived DNA. Improving the yield of all RNA experiment products and the mRNA integrity of IVT products. Suitable for almost all experiments sensitive to RNA integrity.



Product Information

Item No.	Name	Specification
KNB9005	RNase Inhibitor (Recombinant)	2.5 KU, 10 KU

DNase I

Product Information

This product is the DNase I recombinantly expressed in E.*coli*. This endonuclease is capable of non-specific shearing of single-stranded or double-stranded DNA and producing second and third oligonucleotide products. Free of RNase, it guarantees the high purity of DNase I and the integrity of RNA.

Product Composition

Item No.	Composition	Storage Temperature (℃)	Product ID / Specification	
			KNB006S（1 KU）	KNB006L（5 KU）
KNB9006-I	DNase I (RNase-free) (2 U/ul)	-20	0.2 ml	1 ml
KNB9006-II	10X Reaction Buffer	-20	1.5 ml	15 ml

1X Reaction Buffer contains: 10 mM Tris-HCl (pH7.6).

Product Properties

Optimal pH Range: 7–8

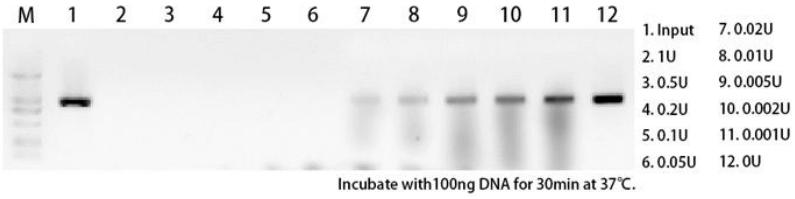
Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to completely degrade 1 ug of pBR322 DNA in 10min at 37°C .

Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100 pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤ 10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

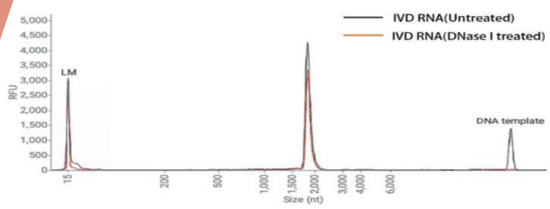
Product Features

High enzyme activity; efficient removal of DNA with microamount.



Incubate with100ng DNA for 30min at 37℃.

Efficient removal of IVT DNA template; no RNase activity; no impact on mRNA integrity.



Product Information

Item No.	Name	Specification
KNB9006	DNase I (RNase-free)	1 KU, 5 KU

RNase III

Product Introduction

This product is the RNase III recombinantly expressed in E.*coli*. This specific exonuclease is capable of cutting double-stranded RNA (dsRNA) and generating 12-35 bp dsRNA fragments with protruding 5'-PO_4, 3'-OH, and 3' termini.

Product Composition

Item No.	Composition	Storage Temperature (°C)	Product ID / Specification	
			KNB9007S (200 U)	KNB9007L (2000 U)
KNB9007-I	RNase III (2 U/ul)	-20	0.5 ml	5 ml
KNB9007-II	10X Reaction Buffer	-20	1.5 ml	15 ml
KNB9007-III	10X EDTA	-20	1 ml	10 ml
KNB9007-IV	10X $MnCl_2$	-20	1 ml	10 ml

10X Reaction buffer contains 500mM Tris-HCl (pH7.5) 500mM NaCl and 10mM DTT.

Product Properties

Optimal Reaction Temperature: 37°C

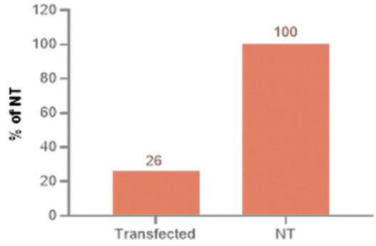
Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to degrade 1 ug of dsRNA into siRNA in 20min at 37°C in a 50 ul reaction system.

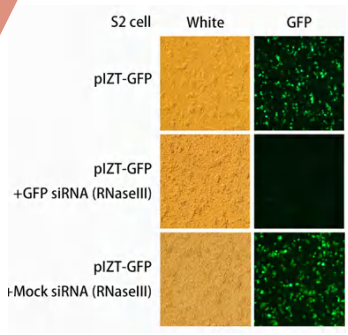
Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

Product Features

Efficient production of siRNA; accurate interference with target gene expression.





Product Information

Item No.	Name	Specification
KNB9007	RNase III	200 U, 2000 U

T4 RNA Ligase

Product Introduction

This product is the ATP-dependent T4RNA Ligase I recombinantly expressed in E. coli. It can catalyze oligonucleotide, single-stranded RNA and DNA intermolecular/ intramolecular 5'-PO_4 and 3'-OH to form phosphodiester bond.

Product Composition

Item No.	Composition	Storage Temperature (℃)	Product ID / Specification	
			KNB9008S (1 KU)	KNB9008L (10 KU)
KNB9008-I	T4 RNA Ligase (10 U/ul)	-20	0.1 ml	1 ml
KNB008-II	10X Reaction Buffer	-20	1.5 ml	15 ml
KNB9008-III	Adenosine-5'-Triphosphate (ATP 10mM)	-20	0.2 ml	2 ml
KNB9008-IV	PEG 8000	-20	1 ml	10 ml

1X Reaction buffer contains 50mM Tris-HCL (pH7.5), 10mM $MgCl_2$ and 1mM DTT.

Product Properties

Optimal Reaction Temperature: 37℃

Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to convert 1nM 5'-[^{32}P]rA_{16} into anti-phosphoric acid form in 30 min at 37℃ .

Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100pg/mg, residual host cell protein ≤ 50ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

Product Usage

- RNA 3' terminus labelling (using cytidine-3',5'-[α-^{32}] diphosphate)
- Connecting RNAs
- Synthesizing oligoribonucleotide and oligodeoxyribonucleotide
- Specific modification of tRNA
- Connecting oligodeoxyribonucleotide to single-stranded cDNA to realize 5'RACE (rapid amplification of cDNA termini)
- Site-specific generation of multiplex PCR primer

Product Information

Item No.	Name	Specification
KNB9008	T4 RNA Ligase	1 KU, 10 KU

Pyrophosphatase Inorganic (yeast)

Product Introduction

This product is the Pyrophosphatase Inorganic recombinantly expressed in E.*coli*. After binding to magnesium ions, it catalyzes the hydrolysis of inorganic pyrophosphate to produce phosphate, and avoids its inhibition of the reaction system, thereby improving the yield of in vitro transcription of RNA.

Product Composition

Item No.	Composition	Storage Temperature (℃)	Product ID / Specification	
			KNB9009S (10 U)	KNB9009L (50 U)
KNB9009-I	Pyrophosphatase Inorganic (yeast) (0.1 U/ul)	-20	100 ul	500 ul

Product Properties

Optimal Reaction Temperature: 25℃

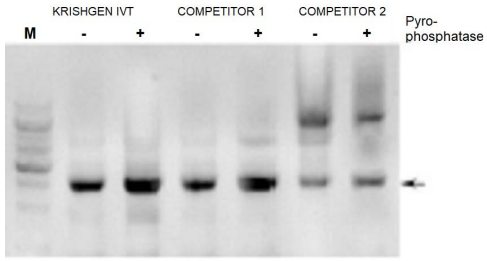
Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to hydrolyze inorganic pyrophosphate and produce 1 umol of phosphate in 1 min under standard reaction conditions (standard reaction conditions: in 10 min at 25℃ , 0.5 ml reaction system containing 100 mM Tris HCl, pH7.2, 2mM $MgCl_2$ and 2mM inorganic pyrophosphate).

Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100 pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

Product Features

Effectively increasing the RNA yield of in vitro transcription.



Product Information

Item No.	Name	Specification
KNB9009	Pyrophosphatase Inorganic (yeast)	10 U, 50 U

Alkaline Phosphatase

Product Introduction

This product is the Alkaline Phosphatase recombinantly expressed in E. coli. It is capable of non-specific catalysis of the dephosphorylation of the phosphomonoester bond of DNA and RNA 5' and 3' termini, as well as catalysis of the dephosphorylation of NTPs and dNTPs.

Product Composition

Item No.	Composition	Storage Temperature (℃)	Product ID / Specification	
			KNB9010S (0.5 KU)	KNB9010L (2.5 KU)
KNB9010-I	Alkaline Phosphatase (10 U/ul)	-20	100 ul	500 ul
KNB9010-II	Reaction Buffer	-20	1.5 ml	15 ml

1X Reaction buffer contains 50mM Bis-Tris-Propane-HCL, pH6, 1mM $MgCl_2$ and 0.1mM $ZnCl_2$.

Product Properties

Optimal Reaction Temperature: 37°C

Definition of Active Unit: 1 active unit is defined as the amount of enzyme needed to de-phosphorylate 1 ug of pUC19 plasmid vector in 30m at 37°C (dephosphorylation is defined as > 95% inhibitory action on re-cyclization in the self-linking reaction, and measured through transformation into E.*coli*.)

Quality Control

Purity ≥ 95%, residual host cell DNA ≤ 100 pg/mg, residual host cell protein ≤ 50 ppm, residual endotoxin ≤10 EU/mg, no residual RNase, endonuclease, exonuclease or protease, germ-free, pathogen-free.

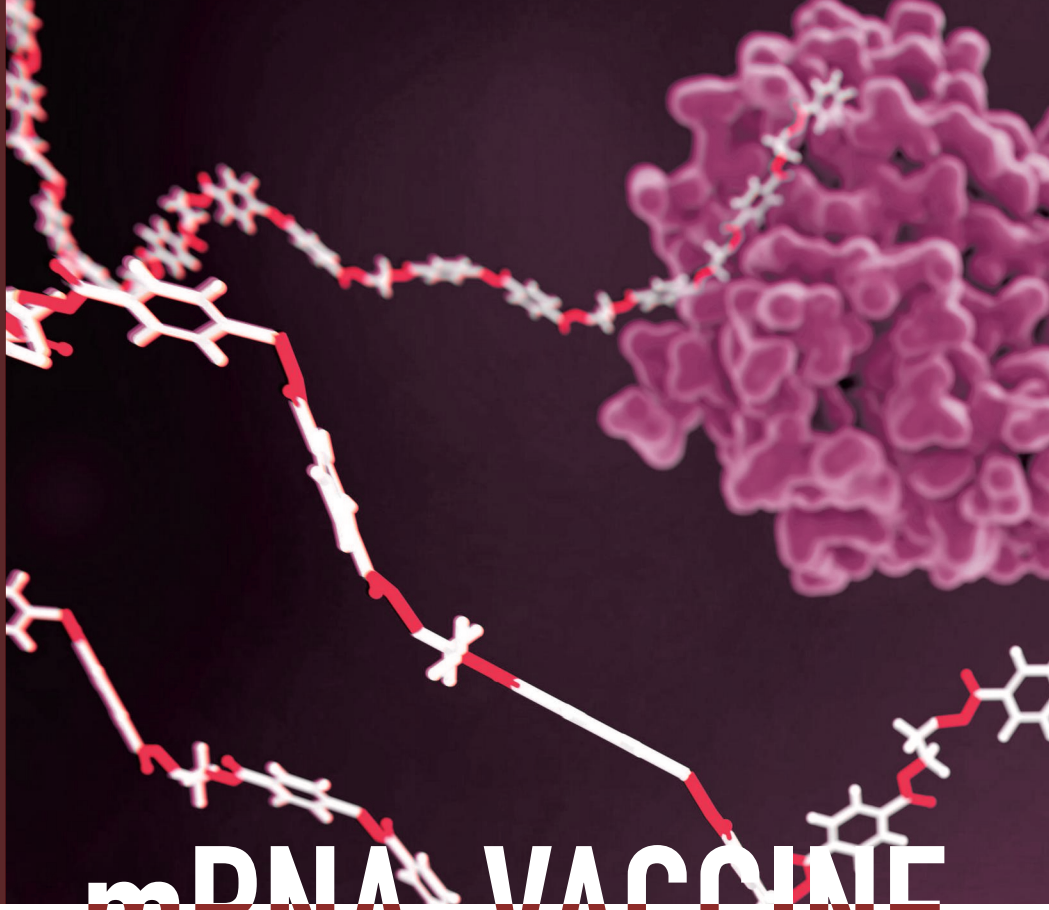
Product Features

Dephosphorylation of DNA and RNA 5' and 3' termini. Can be used for DNA dephosphorylation before terminus-labelling by T4 polynucleotide kinase. Used for treatment of dNTPs in PCR reactions prior to sequencing or SNP analysis. Reducing the immunogenicity of mRNA in mammals by dephosphorylating mRNA. Avoiding re-cyclization during the cloning process by dephosphorylating DNA cloning vector. No BSA system, less heat source contamination.

Product Information

Item No.	Name	Specification
KNB9010	Alkaline Phosphatase	0.5 KU, 2.5 KU

Enzymes Program for



mRNA VACCINE

KRISHGEN BioSystems

OUR REAGENTS. YOUR RESEARCH.

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