DUONING 多宁生物

One-stop solution for mRNA *in vitro* synthesis

Facilitating the industrial production of mRNA vaccines

Product Catalog

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mRNA vaccine

Product Description

As early as more than 30 years ago, VicalIncorporated first used mRNA for drug development, delivered mRNA to the body through liposomes, and found that it could be translated *in vivo*. This study laid a theoretical foundation for the application of mRNA in the field of biopharmaceutical. With the outbreak of COVID-19 pandemic in 2020, mRNA vaccines have ushered in a new era of vaccines, and major pharmaceutical companies domestic and abroad are rushing into the mRNA technology track.

The working principle of the mRNA vaccine is to encapsulate the mRNA fragments of the virus into special lipid nanoparticle, inject them into human body to generate antigens, and then stimulate a specific immune response to achieve the effect of forming immune memory.

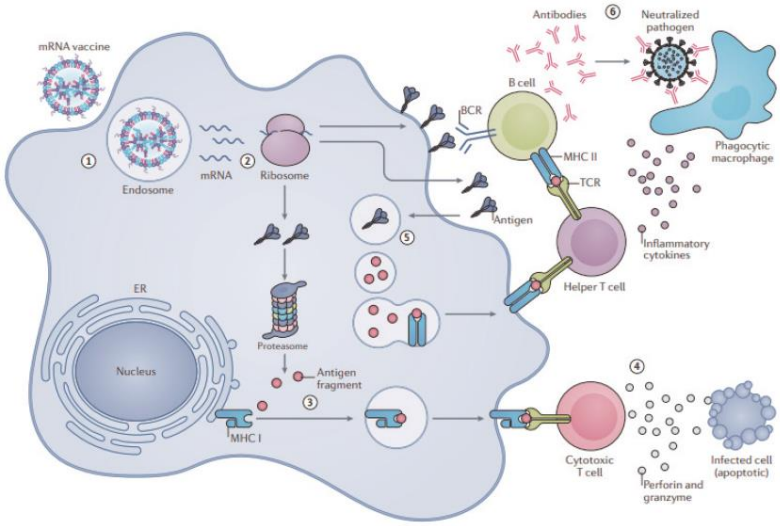


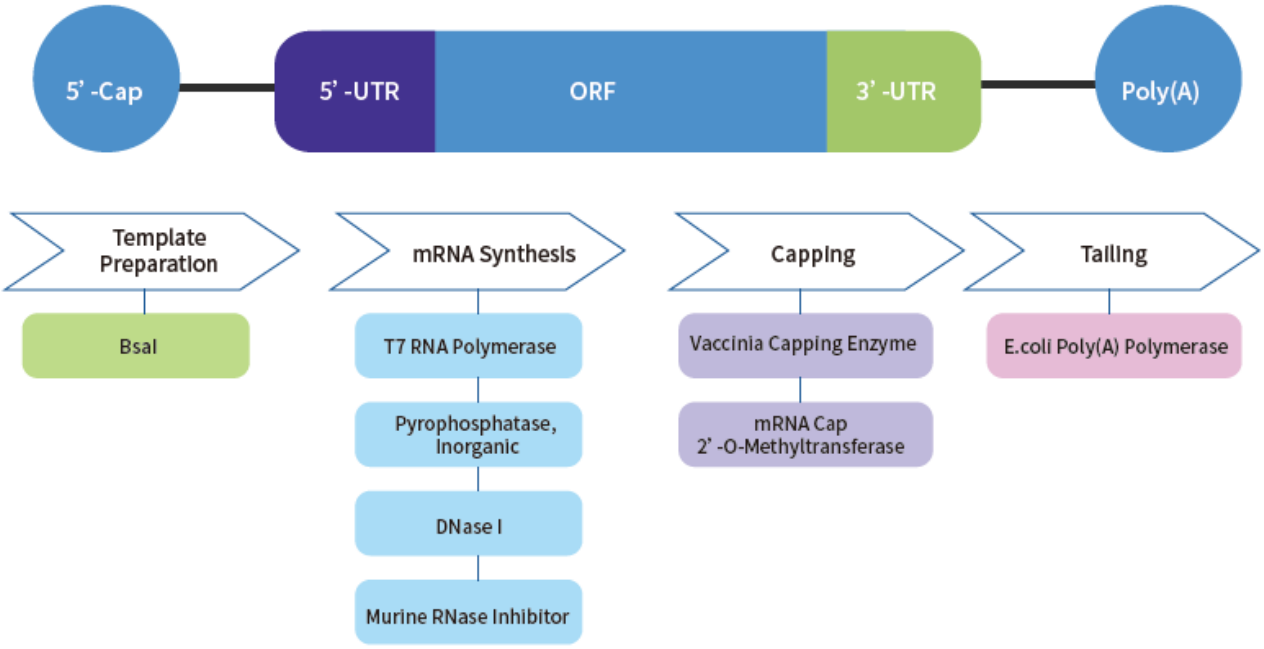
Fig.1 The working mechanism of mRNA vaccine. This figure is quoted from Chaudhary N , Weissman D , Whitehead K A . mRNA vaccines for infectious diseases: principles,delivery and clinical translation[J]. Nature Reviews Drug Discovery, 2021.

Features of mRNA vaccines

- mRNA does not enter the nucleus, and will not be integrated into the human genome, provides high safety;
- Can activate the dual immune system of humoral immunity and cellular immunity, with stronger effectiveness and higher protection rate;
- The development cycle is short, especially when dealing with virus mutations, only need to change the bases of the S protein sequence to quickly develop new candidate vaccines;
- No cell culture and other processes are required, and the production efficiency is higher.

The development of mRNA vaccines includes mRNA synthesis *in vitro*, vector delivery and other processes. Among them, the raw material enzymes required for mRNA synthesis *in vitro* are crucial for the industrial production of mRNA vaccines. Duoning provides a full range of GMP & non-GMP enzymes to facilitate the industrial production of mRNA vaccines.

Duoning's solution of mRNA *in vitro* synthesis

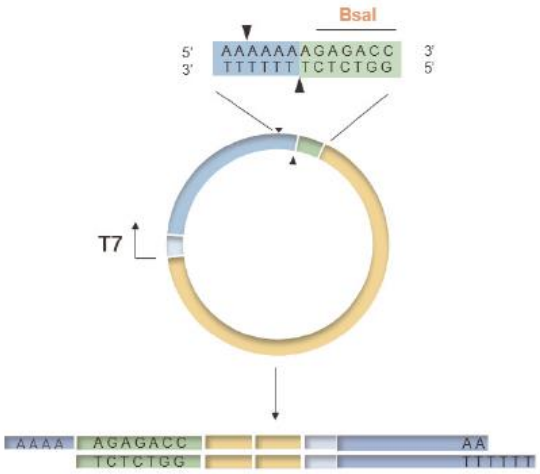


Ordering information

Product name	Part No.	Specification
BsaI (GMP grade)	DNRG0304012-GMP	400 KU
T7 RNA polymerase (GMP grade)	DNRG0304022-GMP	1 MU
Vaccinia capping enzyme (GMP grade)	DNRG0304032-GMP	1 MU
mRNA cap 2'-O-methyltransferase (GMP grade)	DNRG0304042-GMP	5 MU
Inorganic pyrophosphatase (GMP grade)	DNRG0304072-GMP	800 U
Murine Inhibitor (GMP grade)	DNRG0304062-GMP	2.2 MU
DNase I (GMP grade)	DNRG0304052-GMP	40 KU

Linear DNA template preparation

In industrial production, the template for *in vitro* transcription of mRNA is usually plasmid, which needs to be linearized before *in vitro* transcription, because the transcription reaction will continue to the end of the DNA template, and the circular plasmid template will be transcribed to generate mRNA transcripts of different sizes, Linearization ensures mRNA transcripts of defined length and sequence.



Related products

BsaI

Linearized templates can be obtained by digesting plasmids with restriction endonucleases, and it is necessary to ensure that the digested products are blunt-ended or 5'-end protruding structures. Type IIS restriction endonuclease is required. The restriction endonuclease cutting site of type IIS restriction endonuclease is outside the recognition site, and the 5' end protrudes after digestion. BsaI is a type IIS restriction endonuclease. Its recognition site is composed of 6 bases. It is highly specific and extremely difficult to appear 'like' enzyme cutting sites, which can effectively avoid non-specific enzyme cutting.

Product data

Strong enzyme cleavage activity of BsaI

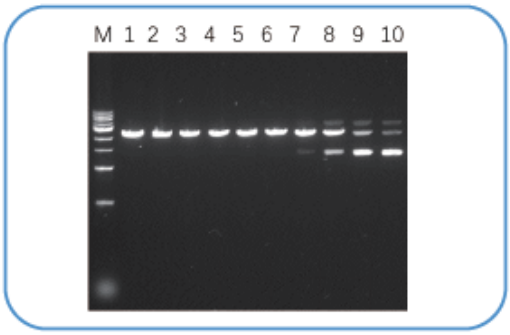


Fig.2 BsaI activity assay. Add 1ug of plasmid and 1ul of BsaI to 20ul system (1 well is stock solution, 1-10 wells are serially diluted 2-fold), and react for 30 minutes.

React for 16h, no star activity

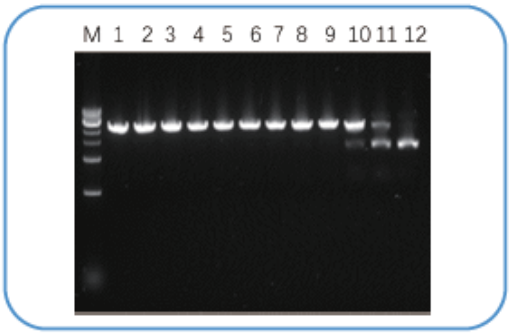


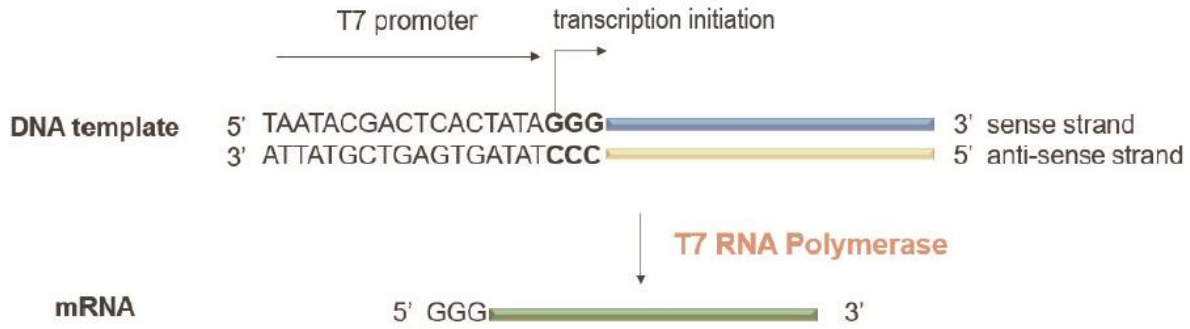
Fig.3 BsaI star activity assay. Add 1 μg of plasmid and 1 μl of BsaI (13 is stock solution, 1-12 wells were serially diluted 2-fold) to the 20ul system, and reacted for 16 hours.

Ordering information

Product name	Part No.	Specification
BsaI (GMP grade)	DNRG0304012-GMP	400KU

mRNA in vitro transcription

In vitro transcription (IVT) uses a linearized plasmid as template to mimic the *in vivo* transcription process to generate mRNA under conditions with RNA transcriptase and NTP. The promoter usually used in the plasmid template is the T7 promoter, which has a high transcriptional intensity and is currently the most widely used promoter for prokaryotic expression. T7 RNA Polymerase is highly specific to the T7 promoter and has high activity. With the assistance of inorganic pyrophosphatase, murine RNase inhibitor, and DNaseI, it can synthesize high-yield mRNA.



Related products

T7 RNA polymerase

This product is a protein encoded by T7 phage DNA expressed by recombinant *E.coli*. It is a DNA-dependent 5'→3' RNA polymerase that highly specifically recognizes the T7 promoter sequence (5'-TAATACGACT CACTATAGGG-3'). Use the single-stranded or double-stranded DNA containing the T7 promoter sequence as a template and NTP as a substrate to synthesize RNA complementary to the single-stranded DNA or double-stranded DNA template strand downstream of the promoter.

Product data

Highly efficient transcription of mRNAs of various lengths

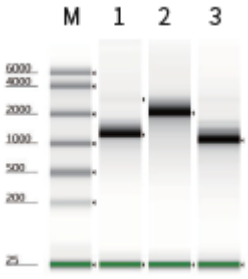


Fig. 4. T7 RNA polymerase transcription efficiency assay. In a 20 μL system, 3 plasmids (1 with poly A tail, 2 and 3 without poly A tail) were used as templates, reacted at 37°C for 2 hours, and the transcription length were 2000nt, 4000nt, and 2000nt.

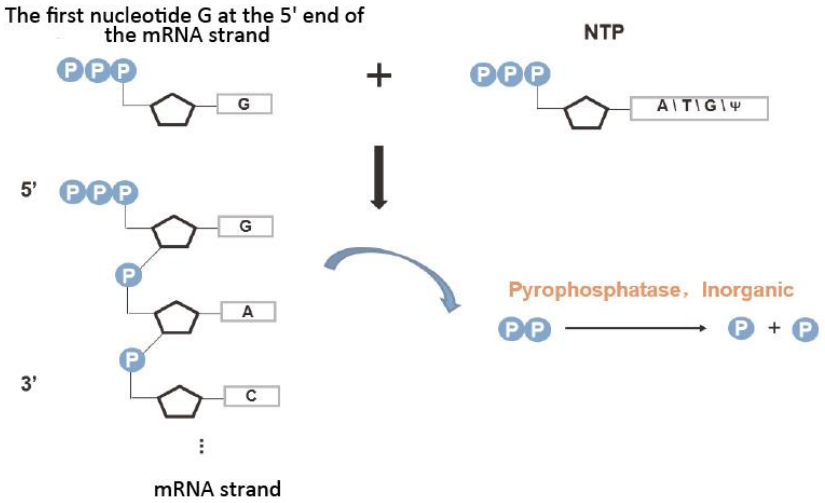
Ordering information

Product name	Part No.	Specification
T7 RNA polymerase (GMP grade)	DNRG0304022-GMP	1MU

Inorganic pyrophosphatase

This product is an inorganic pyrophosphatase (PPase) derived from yeast expressed in recombinant *Escherichia coli*. It is an enzyme that catalyzes the conversion of one ion of pyrophosphate to two phosphate ions, and hydrolyzes inorganic pyrophosphate to generate orthophosphate, which is a highly exothermic reaction, therefore, this reaction can be coupled to thermodynamically unfavorable transformations in order to drive them to completion. It can be used in molecular biology to increase mRNA production in *in vitro* transcription reactions.

Working principle of inorganic pyrophosphatase



Product data

Inorganic pyrophosphatase can effectively increase the yield of mRNA

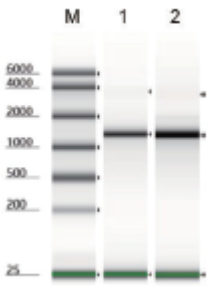


Fig.5 Inorganic pyrophosphatase can effectively increase the yield of mRNA. Additional inorganic pyrophosphatase was added to the 20ul in vitro transcription system, no pyrophosphatase was added to 1 well, and pyrophosphatase was added to another well.

Ordering information

Product name	Part No.	Specification
Inorganic pyrophosphatase (GMP grade)	DNRG0304072-GMP	800U

Murine RNase Inhibitor

This product is a murine RNase inhibitor expressed and purified in soluble form in *Escherichia coli*, which can inhibit the activity of RNase A, RNase B or RNase C, and is mainly involved in the protection of mRNA during *in vitro* transcription of mRNA.

Product name	Part No.	Specification
Murine RNase Inhibitor (GMP grade)	DNRG0304062-GMP	2.2MU

DNaseI (Deoxyribonuclease I)

DNase I is an endodeoxyribonuclease that can digest single-stranded or double-stranded DNA. It recognizes and cleaves phosphodiester bonds to produce 5'-phosphate groups and 3'-OH monodeoxynucleotides or mononucleotides. stranded or double-stranded oligodeoxynucleotides.

Product data

DNaseI enzyme activity is high, can efficiently digest DNA

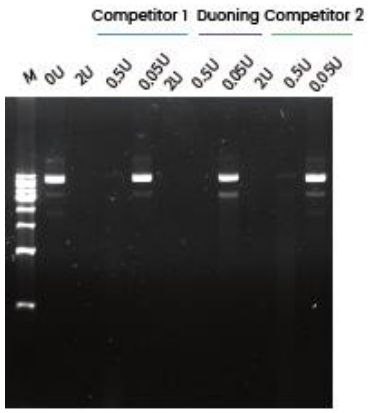


Fig.6 DNaseI digestion effect of DNA. Adding 1ug of DNA and different amounts of DNaseI to the 20ul system, compared with competing products 1 and 2, the digestion performance of D DNaseI is better.

Ordering information

Product name	Part No.	Specification
DNase I (GMP grade)	DNRG0304052-GMP	40KU

mRNA capping system

The 5' end of the mRNA transcribed *in vitro* contains a triphosphate group, which is highly immunogenic. If it is delivered into the body, it will activate the innate immune response, and RIG-1 will recognize the triphosphate group, making it unable to produce the correctly translated antigenic protein. Therefore, mRNA needs to be capped to evade the innate immune system in industrial production. Enzymatic capping, first capping the 5' end of the mRNA with vaccinia capping enzyme, and then further using mRNA cap 2'-O-methyltransferase (GMP grade) to convert the cap0 to cap1. Cap 1 is less immunogenic than cap 0 and is not easily recognized by RNA sensors.

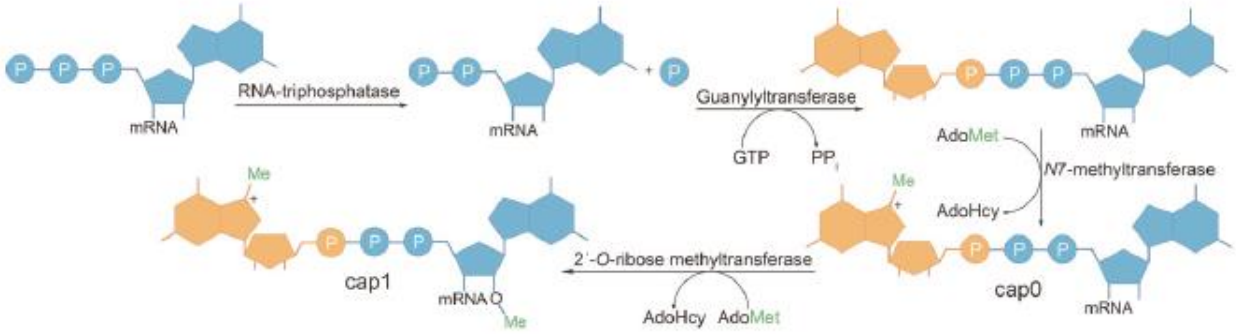
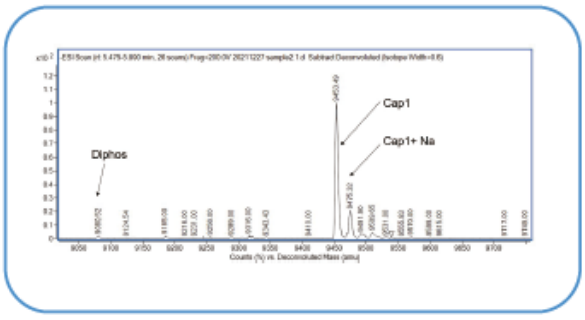


Fig.7 mRNA enzymatic capping. This figure is quoted from Fabian M, Nils M, Andrea R. Synthetic mRNA capping[J]. Beilstein Journal of Organic Chemistry, 2017, 3, 4. Ground for 19-2832. Note: Vaccinia Capping Enzyme performs the functions of three enzymes: RNA-triphosphatase, Guanylytransferase, and N7-methyltransferase.

Product data

LC-MS platform detects the capping rate of cap1, and the capping rate exceeds 99%



	Mass	Area	%
Cap1	9453.4903	29019369	99.01%
Cap1+Na	9475.3221	9988746	99.01%
5' -monophosphate	/	/	/
5' -diphosphate 5'	9080.5199	390758	0.99%
triphosphate	/	/	/

Product name	Part No.	Specification
Vaccinia capping enzyme (GMP grade)	DNRG0304032-GMP	1MU
mRNA cap 2'-O-methyltransferase (GMP grade)	DNRG0304042-GMP	5MU

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