Reagents and Complete Solutions for mRNA-based Drug Development

www.yeasenbiotech.com

Company Overview

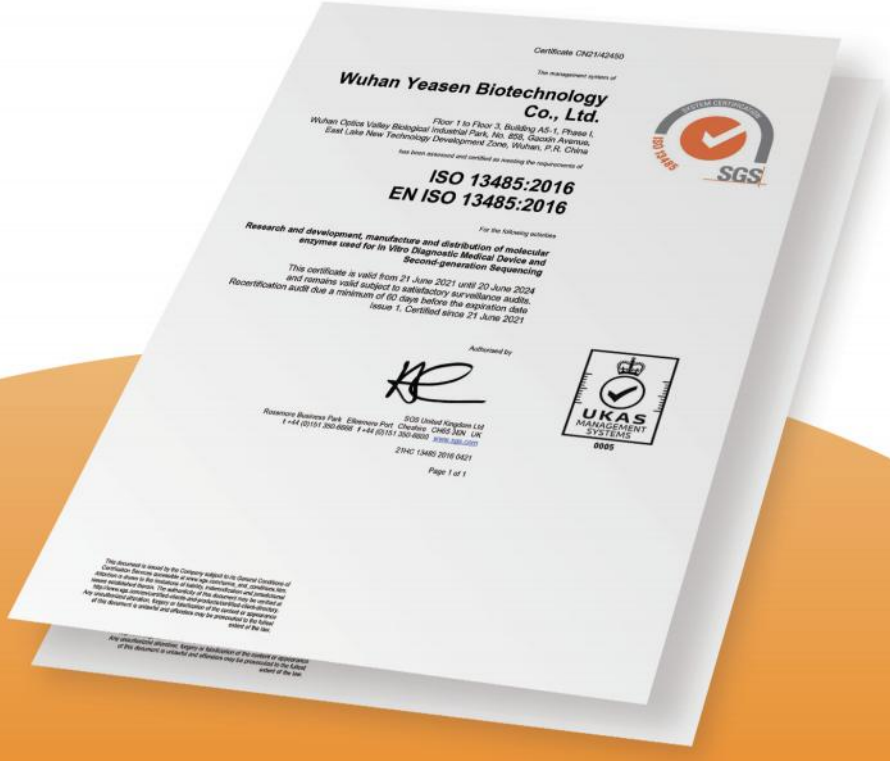
Founded in 2014, Yeasen Biotechnology Co.,Ltd is a high-tech enterprise committed to the development and production of enzymes and antibodies for the life science industry. Products supplied by Yeasen include molecular diagnostic enzymes, proteins and antibodies applicated in the industry of medicine, food safety testing, breeding and judicature. Yeasen is dedicated to providing our customers with high-quality products and services.

Contents

Yeasen Provides GMP-grade Reagents

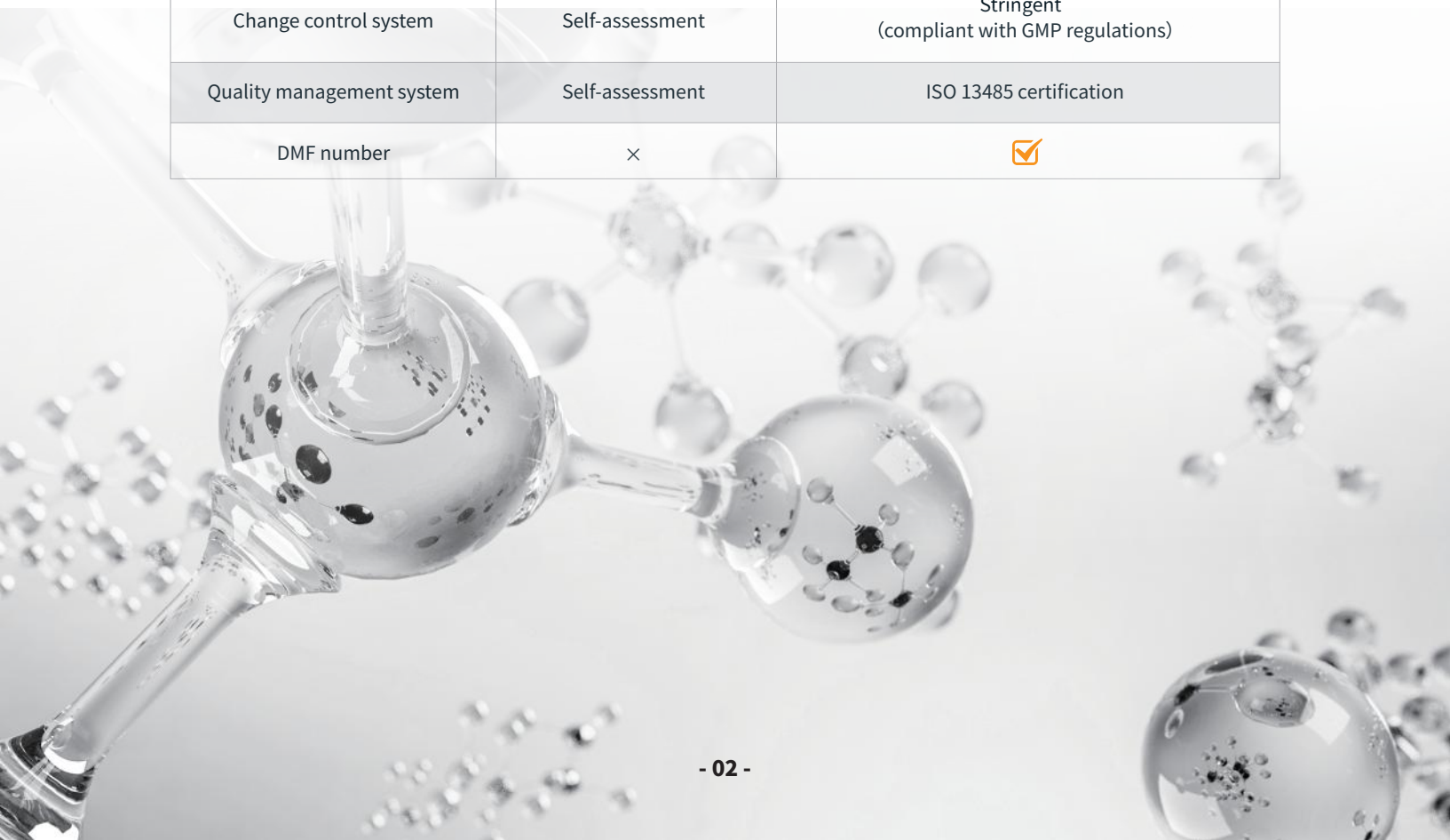
Yeasen is the first company receiving the ISO 13485 certificate for research, development, manufacture and distribution of molecular enzymes in China. “ISO” stands for International Organization for Standardization. “13485” refers to the specific certification for medical devices and ancillary products. ISO 13485 specifies requirements for the quality management system where an organization needs to demonstrate its ability to provide medical devices and related services that consistently meet customer and applicable regulatory requirements. The ISO 13485 certification therefore ensures quality, consistency, and traceability. Yeasen has successfully achieved the ISO 13485 certification and we undergo a successful inspection every year.

“GMP-grade” is a branding term that Yeasen uses to describe reagents manufactured at ISO 13485 certified facilities. Yeasen’ s GMP-grade reagents are produced in compliance with ISO 13485 quality management system standards and with more stringent process controls and complete documentation records. Yeasen can not only offer high-quality reagents, but also provide documents, site audit and other support to our customers.



Why to Choose GMP-grade Raw Materials?

Quality Controls	Research-grade	GMP-grade
Animal-free	× Animal-derived material may be used	☑ (Animal-free certificates can be provided if required)
Cell bank characterization	Less stringent	Stringent (compliant with GMP regulations)
Traceability information for raw materials and finished product	Less stringent	Stringent (compliant with GMP regulations)
Endotoxin level control	×	☑ Stringent
Sterile control	×	☑ Stringent
Batch records for manufacturing and testing	Less stringent	Stringent (Batch records can be provided if required)
Process validation report	×	☑ (The report can be provided if required)
Change control system	Self-assessment	Stringent (compliant with GMP regulations)
Quality management system	Self-assessment	ISO 13485 certification
DMF number	×	☑



Yeasen Acquired DMF Numbers for Many Products

Drug master files (DMFs) are documents submitted voluntarily to the US Food & Drug Administration (FDA) that contain confidential, detailed information about facilities, processes, or articles used in the manufacturing, processing, packaging, and storing of human drug products.

The FDA reviews the technical contents of a DMF when an API manufacturer incorporates it as a reference to support its Investigational New Drug Application (IND), New Drug Application (NDA), an Abbreviated New Drug Application (ANDA), and Export Application. DMFs have significant roles in the application. In fact, the FDA approval process is significantly shortened when using raw materials of manufacturers that have filed DMFs for these specific materials.

Yeasen has submitted DMFs to FDA and acquired corresponding DMF numbers for many products. You may request that we provide reference authorization to our DMFs in support of your application with FDA. To initiate the reference authorization of our DMFs, please submit a Letter of Authorization request to Yeasen requesting that we provide a DMF Letter of Authorization to the respective FDA Center. Wherever you are in the world, contact us at overseas@yeasen.com to reference our DMFs.

The mRNAtools Facility

Based on wide experience and technical advantages in the enzyme development and industrial production, Yeasen constructed a new facility, named as “mRNAtools”, to supply raw materials for mRNA-based drugs. Covering 50000 square feet, the mRNAtools facility is built and operated in accordance with GMP regulations and is equipped with 2×1500L automatic fermentation equipment, industrial-scale purification and lyophilization equipment.

Up to now, the mRNAtools facility has been applied to the production of raw materials required in the manufacture of mRNA-based drugs. These raw materials are manufactured in compliance with the ISO 13485 QMS standards and GMP regulations, satisfying the production and registration requirements of customers.

Workflow & Reagents

Yeasen can supply all raw materials required in mRNA synthesis

Template Generation

Plasmid Linearization

- BspQI
- BsaI
- XbaI

In Vitro Transcription

IVT

- T7 High Yield RNA Synthesis Kit
- T7 RNA Polymerase
- T7 RNA Polymerase Mix
- Pyrophosphatase, Inorganic
- Murine RNase Inhibitor
- DNase I
- 10×Transcription Buffer
- Natural and Modified Nucleoside Triphosphates

mRNA Modification

mRNA Capping

- Vaccinia Capping Enzyme
- 2'-O-Methyltransferase
- Cap Analogs
- S-adenosylmethionine (SAM)
- 10×Capping Buffer

Poly(A) Tailing

- Poly(A) Polymerase

mRNA Purification

Purification

- mRNA Isolation Master Kit
- RNA Cleaner
- Magnetic Separation Rack

Template Generation

Plasmid linearization is an indispensable step during template generation when plasmid vectors are used as transcription templates. As transcription proceeds to the end of DNA templates, plasmid linearization ensures that RNA transcripts of a defined length and sequence are generated.

Yeasen provides various restriction enzymes to meet your needs. We recommend selecting restriction enzymes that generate blunt ends or 5´-overhangs.

Products

Product	Cat. No.	Cleavage Site
BspQ I	10664ES	5' ——— GCTCTTC(N)↓ ——— 3' 3' ——— CGAGAAG(NNNN) ↑ ——— 5'
BsaI	10661ES	5' ——— GGTCTC(N) ↓ ——— 3' 3' ——— CCAGAG(NNNNN) ↑ ——— 5'
XbaI	10662ES	5' ——— T↓CTAGA ——— 3' 3' ——— AGATC↑T ——— 5'

GMP-grade **High Purity** **High Specific Activity**

Applications

Example: Restriction digestion

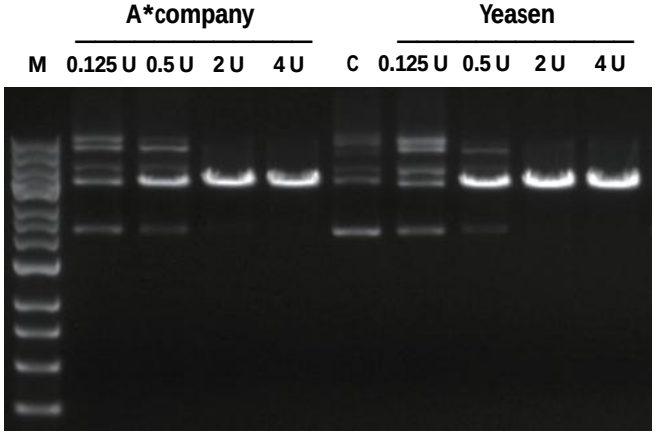


Figure1. The performance of Yeasen BspQI is superior.

In a 50 μL reaction system, 1 μg of λDNA was treated with the corresponding amount of BspQI (incubate at 50 °C for 60 min and then incubate at 80 °C for 20 min to inactivate BspQI). Then 20 μL of the reaction solution was loaded.

M: DNA marker
C: The control group without BspQI treatment

In Vitro Transcription (IVT)

T7 RNA Polymerase is a DNA-dependent RNA polymerase that is highly specific for the T7 phage promoters. In cell-free system with DNA templates and NTPs, T7 RNA Polymerase catalyzes *in vitro* transcription from a cloned DNA sequence under the T7 promoters to synthesis RNAs.

Products

- T7 High Yield RNA Synthesis Kit
- T7 RNA Polymerase
- T7 RNA Polymerase Mix
- Pyrophosphatase,Inorganic
- Murine RNase Inhibitor
- DNase I
- 10×Transcription Buffer
- ATP
- GTP
- CTP
- UTP
- Pseudo-UTP
- N1-Me-Pseudo-UTP

GMP-grade **High Yield** **High Integrity**

Applications

Example 1: Standard RNA synthesis

A

Component	20 µL Reaction	Final Concentration
10× Transcription Buffer	2 µL	1×
T7 RNA Polymerase Mix	2 µL	-
ATP/GTP/CTP/UTP (100mM each)	2 µL each	10 mM each
DNA Templates	1 µg	-
RNase-free H_2O	2 µL	10 mM

B

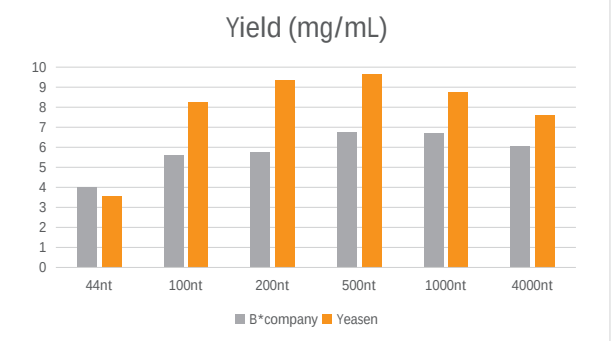


Figure 1. High yield can be achieved by Yeasen standard RNA synthesis reaction.

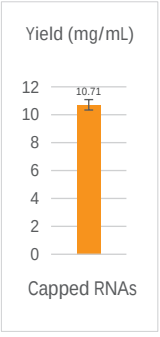
A 20 µL IVT reaction for standard RNA synthesis was set up according to the table (A) and incubated at 37°C for 2 hours in a PCR machine. Transcripts were purified by magnetic beads (RNA Cleaner, Yeasen#12602) and quantified on NanoDrop Spectrophotometer (B). All reaction reagents are from T7 RNA Synthesis Kit (Yeasen#10623 or B* company). The IVT reaction yields of Yeasen and B* company are compared.

Example 2: Capped RNA synthesis with modified nucleotides and cap analogs

A

Component	20 μL Reaction	Final Concentration
10× Transcription Buffer	2 μL	1×
T7 RNA Polymerase	250 U	-
Pyrophosphatase, Inorganic	0.04 U	-
Murine RNase Inhibitor	20 U	-
A/G/C/N1-Me-pUTP (100mM)	2 μL each	10 mM each
Cap1-GAG (100mM)	2 μL	10 mM
DNA Templates	1 μg	-
RNase-free H_2O	Up to 20 μL	-

B



C

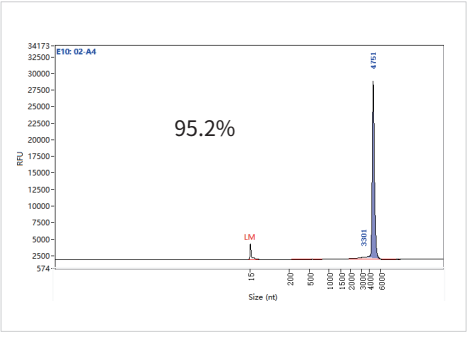


Figure 2. High yield and high integrity can be achieved by Yeasen capped RNA synthesis reaction.

A 20 μL IVT reaction for capped RNA synthesis was set up according to the table (A) and incubated at 37°C for 2 hours in a PCR machine. Transcripts were purified by magnetic beads (RNA Cleaner, Yeasen#12602) and quantified by NanoDrop® spectrophotometer (B). The length and integrity of transcripts was assessed by capillary electrophoresis (C).

More Information about Nucleotides

Nucleotides are critical building blocks for producing revolutionary mRNA-based medicines. Yeasen is devoted to being a leader in the manufacture and supply of mRNA building blocks—nucleotides and modified nucleotides.

Yeasen offers the mRNA building blocks conformed to the following attributes and specification:

Attributes

- Validated, product-specific process and analytical methods
- Product-specific stability
- Documentation follows applicable GMP guidelines
- AOF production process and raw materials (TSE & BSE)
- Nitrosamine statement
- Regulatory support documents available
- Large-scale production (grams to kilograms)
- Standard nucleotide products always in the sodium salt form (Na+), customized products in different salt forms (NH4+, Tris etc) also available to meet different downstream application needs

Specification

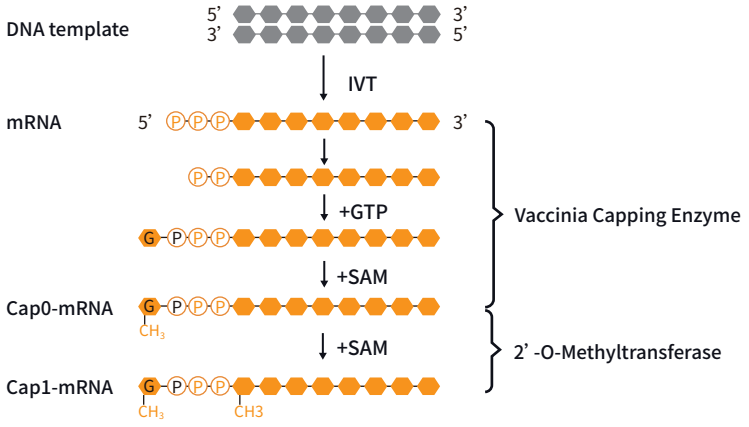
Item	Nucleotides/ Modified Nucleotides
Appearance	Conform
Purity (HPLC)	⩾99%
^{1}H NMR(D_2O)	Conform
^{31}P NMR(D_2O)	Conform
pH	7.0 ± 0.2
Content	100mM ± 3mM
Bacterial Endotoxin	⩽1.0EU/ml
Exonuclease	Pass
Nickase	Pass
	RNase

mRNA Capping

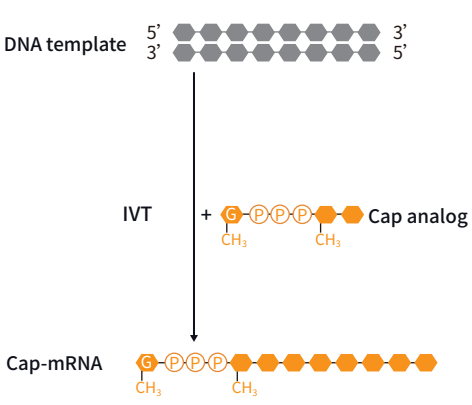
As 5´ cap structure reduces immunogenicity and is required for efficient translation of mRNAs, adding cap structures to the 5′ end of mRNA generated by *in vitro* transcription is necessary.

Capping Strategies

Post-transcriptional Capping



Co-transcriptional Capping



Products

- Vaccinia Capping Enzyme
- S-adenosylmethionine (SAM)
- 2'-O-Methyltransferase
- 10×Capping Buffer

Products

- Cap Analogs

GMP-grade | **High Purity** | **High Capping Efficiency**

Applications

Example 1: Post-transcriptional Capping

A

Component	20 µL Reaction	Final Concentration
Denatured RNA	10 µg	0.5 µg/µL
10× Capping Buffer	2 µL	1×
GTP (10 mM)	1 µL	0.5 mM
SAM (10 mM, fresh)	1 µL	0.5 mM
Murine RNase Inhibitor	20 U	1 U/µL
Vaccinia Capping Enzyme	50 U	2.5 U/µL
2´-O-Methyltransferase	50 U	2.5 U/µL
RNase-free H_2O	Up to 20 µL	-

B

	Percentage(%)
Cap1	99.03
Cap0	0.21
G-Cap	0.14
pp-RNA	0.62
ppp-RNA	0

Figure 1. The capping efficiency of Yeasen post-transcriptional capping reaction could be close to 99%.

10 µg RNAs were denatured by incubation at 65°C for 5 min before capping. A 20 µL post-transcriptional capping reaction was set up according to the table (A) and incubated at 37°C for 2 hours in a PCR machine. Transcripts were purified by magnetic beads (RNA Cleaner, Yeasen#12602). Then the capping efficiency is detected by LC-MS (B).

Example 2: Co-transcriptional Capping

	Cap1	ppp-RNA
Percentage(%)	98.93	1.07

Figure 2. The capping efficiency of Yeasen co-transcriptional capping reaction could be close to 99%.

A 20 µL co-transcriptional capping reaction was set up according to the table (Page8,Figure 2A) and incubated at 37°C for 2 hours in a PCR machine. Transcripts were purified by magnetic beads (RNA Cleaner, Yeasen#12602). Then the capping efficiency is detected by LC-MS.

mRNA Purification

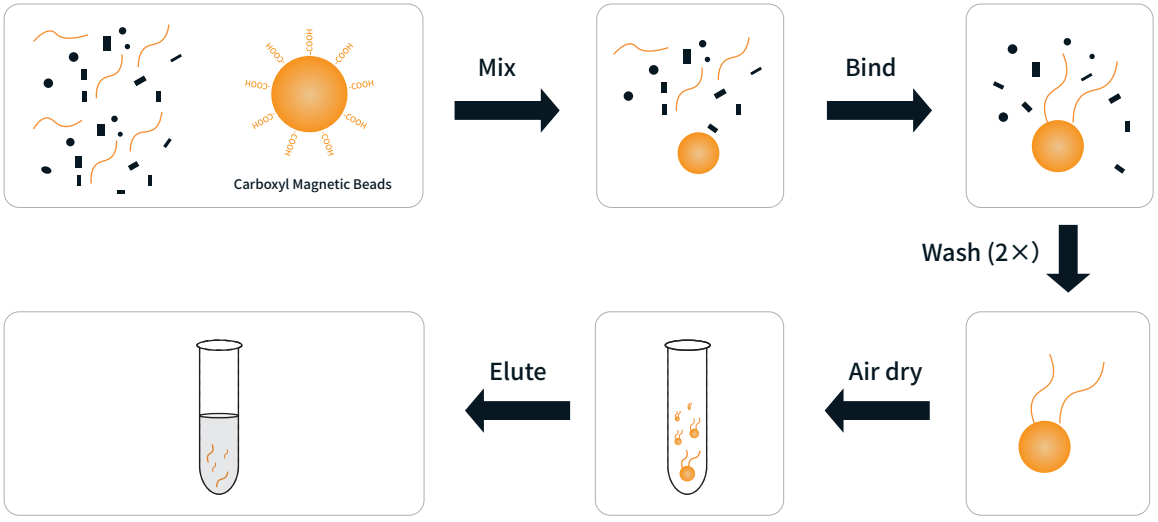
Purification is an indispensable step to achieve high-purity mRNAs.

Products

- **RNA Cleaner**
- **Magnetic Separation Rack**

High Efficiency | **High Quality** | **Wide Applicability**

Capture Workflow



FAQs

1. The yield of IVT reaction is low.

The template quality is closely related to the yield. If the yield of the experimental group is significantly lower than that of the positive control group, the possible reasons can be:

❶ The templates contain components that will inhibit IVT reaction.

❷ There is something wrong with templates.

Suggestions:

❶ Re-purify templates.

❷ Confirm the concentration and integrity of templates.

❸ Extend the reaction time.

❹ Increase the input of templates.

❺ Try other RNA polymerases and corresponding promoters.

2. The yield of short transcripts is low.

If the target transcripts are shorter than 100 nt, extend the reacion time to 4-8 hours or increase the input of templates to 2ug in 20 μL reaction system.

3. There are unexpected longer transcripts.

If the result of gel electrophoresis shows that there are unexpected longer transcripts, the possible reasons can be:

❶ Plasmid templates might not be fully linearized.

❷ Templates have cohesive ends with 3' overhangs.

❸ Transcripts have secondary structure that is not completely denatured.

Suggestions:

❶ Check whether plasmid templates are fully linearized. If necessary, perform plasmid linearization again.

❷ Select suitable restriction enzymes to linearize plasmid templates and avoid producing cohesive ends with 3' overhangs. If necessary, it is practicable to use Klenow fragment or T4 DNA polymerase to produce blunt end.

❸ Use denatured gel to detect transcripts.

4. There are unexpected shorter transcripts.

If the result of gel electrophoresis shows that there are unexpected shorter transcripts, the possible reasons can be:

❶ There is sequence analogous to the termination sequence of T7 RNA polymerase in templates.

❷ The GC content of templates is high.

Suggestions:

❶ Decrease the reaction temperature (such as 30°C) but note that sometimes decreasing the reaction temperature will reduce yields.

❷ Try other RNA polymerases to catalyze IVT reaction.

❸ If the GC content of templates is high, set the IVT reaction temperature at 42°C or add SSB into the IVT reaction system to increase yields and the length of transcripts.

5. There is smearing of transcripts during gel electrophoresis.

If there is smearing of transcripts during gel electrophoresis, the possible reasons can be:

❶ There is RNase contamination during experimental operation.

❷ DNA templates are contaminated by RNases.

Suggestions:

❶ Ensure all reagents are formulated with RNase-free H_2O. Use RNase-free pipette tips and Eppendorf tubes and wear disposable latex gloves and masks during experimental operation.

❷ Re-purify DNA templates.

Ordering Information

The following are representative products offered by Yeasen. Additional sizes are available. Our products are highly optimized to work in concert, to help ensure superior performance and reproducibility.

We can also provide customized services. If you' re interested in a product that isn' t shown, contact us and we' ll work with you to meet your needs.

Products

Reaction System	Cat. No.	Product	Specification
Template Generation	10664ES	BspQI GMP-grade (10 U/μL)	500/2500 U/10/100 KU
	10661ES	Bsa I GMP-grade (20 U/μL)	500/2500 U
	10662ES	Xba I GMP-grade (20 U/μL)	500/2500 U
	10667ES	10×Digestion buffer 3 GMP-grade	1/10/50 mL
	10668ES	10×Digestion Buffer GMP-grade	1/10/50 mL
***In Vitro* Transcription**	10623ES	T7 High Yield RNA Synthesis Kit	50/100/500 T
	10625ES	T7 RNA Polymerase GMP-grade (250 U/μL)	10/100/2500 KU/100MU
	10624ES	T7 RNA Polymerase GMP-grade (50 U/μL)	5000/50000 U
	10671ES	T7 RNA Polymerase Mix GMP-grade	40/400 μL/10/400 mL
	10620ES	Pyrophosphatase, Inorganic GMP-grade (1 U/μL)	10/100/1000 U/40 KU
	10621ES	Murine RNase inhibitor GMP-grade (40 U/μL)	10/20/100 KU/1 MU
	10611ES	Deoxyribonuclease I (DNase I) GMP-grade (2 U/μL)	500/2000/10000 U
	10129ES	ATP Solution GMP-grade (100 mM)	1/5/25/500 mL
	10130ES	CTP Solution GMP-grade (100 mM)	1/5/25/500 mL
	10131ES	UTP Solution GMP-grade (100 mM)	1/5/25/500 mL
	10132ES	GTP Solution GMP-grade (100 mM)	1/5/25/500 mL
	10133ES	NTP Set Solution (ATP, CTP, UTP, GTP, 100 mM each)	1 Set (4 vials)
	10650ES	Pseudo UTP sodium solution GMP-grade (100 mM)	20/100/600 μL/1/5 mL
	10651ES	N1-Me-Pseudo UTP sodium solution GMP-grade (100 mM)	20/100 μL/1/5 mL
	10652ES	ATP Tris Solution GMP-grade (100 mM)	1/5/25/500 mL
	10653ES	CTP Tris Solution GMP-grade (100 mM)	1/5/25/500 mL
	10654ES	UTP Tris Solution GMP-grade (100 mM)	1/5/25/500 mL
	10655ES	GTP Tris Solution GMP-grade (100 mM)	1/5/25/500 mL
	10656ES	Pseudo UTP Tris Solution GMP-grade (100 mM)	20/100 μL/1/5 mL
	10657ES	N1-Me-Pseudo UTP Tris Solution GMP-grade (100 mM)	20/100 μL/1/5/25/500 mL
	10627ES	10×Transcription Buffer GMP-grade	1/10/25/500 mL

Products

Reaction System	Cat. No.	Product	Specification
mRNA Capping	10614ES	mRNA Vaccinia Capping Enzyme GMP-grade (10 U/μL)	2/10/100 KU/5 MU
	10612ES	mRNA Cap 2´-O-Methyltransferase GMP-grade (50 U/μl)	10/50/250 KU/20 MU
	10619ES	S-adenosylmethionine (SAM) GMP-grade (32 mM)	0.5/25/50/500 mL
	Inquire	Cap Analogs GMP-grade (100 mM)	Inquire at overseas@yeasen.com
	10666ES	10× Capping Buffer GMP-grade	1/10/25/500 mL
mRNA Purification	12602ES	RNA Cleaner	1/5/60/450 mL
	12603ES	mRNA Isolation Master Kit	24/96 T
	80460ES	PCR Magnetic Separation Rack	1
	80461ES	2 mL Magnetic Separation Rack	1

References

- Vogel, A. B. et al. Self-Amplifying RNA Vaccines Give Equivalent Protection against Influenza to mRNA Vaccines but at Much Lower Doses. Molecular therapy : the journal of the American Society of Gene Therapy 26, 446-455, doi:10.1016/j.ymthe.2017.11.017 (2018).

- Fuchs, A. L., Neu, A. & Sprangers, R. A general method for rapid and cost-efficient large-scale production of 5' capped RNA. RNA (New York, N.Y.) 22, 1454-1466, doi:10.1261/rna.056614.116 (2016).

- Schmid, A. Considerations for Producing mRNA Vaccines for Clinical Trials. Methods in molecular biology (Clifton, N.J.) 1499, 237-251, doi:10.1007/978-1-4939-6481-9_15 (2017).

- Banerji, A. et al. mRNA Vaccines to Prevent COVID-19 Disease and Reported Allergic Reactions: Current Evidence and Suggested Approach. The journal of allergy and clinical immunology. In practice 9, 1423-1437, doi:10.1016/j.-jaip.2020.12.047 (2021).

- Richner, J. M. et al. Modified mRNA Vaccines Protect against Zika Virus Infection. Cell 168, 1114-1125.e1110, doi:10.1016/j.cell.2017.02.017 (2017).

- Corbett, K. S. et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature 586, 567-571, doi:10.1038/s41586-020-2622-0 (2020).

- Corbett, K. S. et al. mRNA-1273 protects against SARS-CoV-2 beta infection in nonhuman primates. Nature immunology 22, 1306-1315, doi:10.1038/s41590-021-01021-0 (2021).

- Keech, C. et al. Phase 1-2 Trial of a SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine. The New England journal of medicine 383, 2320-2332, doi:10.1056/NEJMoa2026920 (2020).

- Kramps, T. & Elbers, K. Introduction to RNA Vaccines. Methods in molecular biology (Clifton, N.J.) 1499, 1-11, doi:10.1007/978-1-4939-6481-9_1 (2017).

- Linares-Fernández, S., Lacroix, C., Exposito, J. Y. & Verrier, B. Tailoring mRNA Vaccine to Balance Innate/- Adaptive Immune Response. Trends in molecular medicine 26, 311-323, doi:10.1016/j.molmed.2019.10.002 (2020).

- Maruggi, G., Zhang, C., Li, J., Ulmer, J. B. & Yu, D. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. Molecular therapy : the journal of the American Society of Gene Therapy 27, 757-772, doi:10.1016/j.ymthe.2019.01.020 (2019).

- Mascola, J. R. & Fauci, A. S. Novel vaccine technologies for the 21st century. Nature reviews. Immunology 20, 87-88, doi:10.1038/s41577-019-0243-3 (2020).

- Pardi, N., Hogan, M. J. & Weissman, D. Recent advances in mRNA vaccine technology. Current opinion in immunology 65, 14-20, doi:10.1016/j.coi.2020.01.008 (2020).

YEASEN

To enable success of our customers
Together to make a healthier and brighter world

Yeasen Biotechnology (Shanghai) Co., Ltd.

www.yeasenbiotech.com

overseas@yeasen.com