FUJIFILM
Value from Innovation

Wako

Reagents for mRNA Synthesis

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Introduction

Challenges of mRNA Therapeutics and Vaccines, and RNA Modifications

mRNA therapeutics and vaccines are mRNA molecules that are designed to express target proteins or antigens in the body for the prevention or treatment of diseases. Like DNA-based gene therapy and DNA vaccines, mRNA therapeutics and vaccines involve expressing proteins that have preventive or therapeutic effects against diseases. The differences are that mRNA does not need to be transported to the cell nucleus, thus reducing the risk of insertion into the genome, and that the time from mRNA administration to protein expression is short because only translation takes place. These are the main advantages of mRNA therapeutics and vaccines. On the other hand, the challenges of mRNA therapeutics and vaccines include the fact that mRNA is unstable and easily degraded, as well as the immunogenicity of mRNA itself. For these reasons, bringing mRNA therapeutics into practical use has faced considerable hurdles.
When administered to the body, mRNA stimulates Toll-like receptors (TLRs) that recognize exogenous nucleic acids of bacterial or viral origin, thereby triggering an inflammatory response. One way to reduce the immunogenicity of mRNA is the use of modified nucleosides. In eukaryotes, modifications to ribose and some of the bases of RNA are known to occur, and more than 100 different RNA modifications have been reported to date. RNA modifications are thought to contribute to conformational stabilization and subcellular localization of RNA, though their roles are not fully understood.
Against this backdrop, in 2005, Karikó and colleagues discovered that TLR-mediated immune responses were reduced by replacing bases constituting mRNA with modified nucleosides such as 5-methylcytidine, N6-methyladenosine, and pseudouridine. In 2008, they reported that the translation efficiency was greatly improved with mRNAs containing pseudouridine. Various modified nucleosides have since been explored, and some of the mRNA vaccines against COVID-19 contain N^1-methyl pseudouridine, which resulted in even higher translational efficiency than pseudouridine-containing mRNA.

mRNA Synthesis Method

An enzymatic method called in vitro transcription is commonly used for the synthesis of long RNAs such as mRNA therapeutics and vaccines. Linear double-stranded DNA is used as a template and transcribed into mRNA using nucleoside triphosphates, the building blocks of mRNA, and the enzyme RNA polymerase. RNA polymerase binds to the promoter region of the DNA and synthesizes mRNA by connecting complementary nucleoside triphosphates one after the other, using one strand of the DNA as a template, as it moves in the direction from the 5' end to the 3' end (Figure 1).

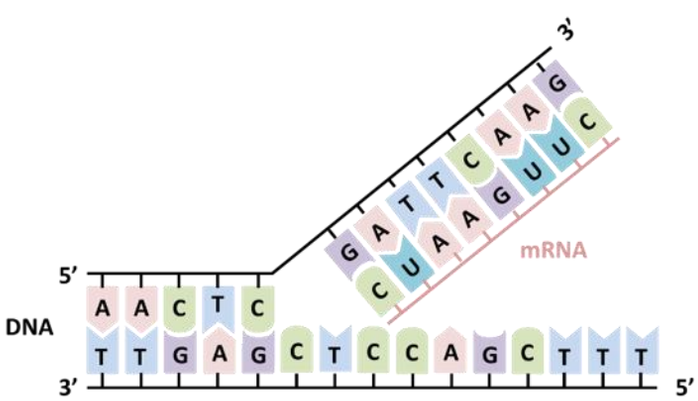


Figure 1. Transcription reaction

Since mRNA synthesized by in vitro transcription reactions does not have the cap structure, it is necessary to create one during the synthesis process. There are two ways to create the cap structure: by using a capping enzyme after transcription, or by using a cap analog such as Anti-Reverse Cap Analog (ARCA) during the transcription. With the method using ARCA, both transcription and capping can be performed in one step, and the cap structure is easily created. The low capping efficiency, however, has been a challenge. To overcome this problem, new capping analogs with improved capping efficiency have been developed.

Preparation of Template DNA

For mRNA synthesis by *in vitro* transcription, high-quality DNA is first required as a template.
The template used in *in vitro* transcription is typically double-stranded DNA, such as PCR products or plasmid DNA linearized by restriction enzyme digestion. FUJIFILM Wako offers a range of reagents required for the preparation of template DNA, including reagents for preparing PCR products and plasmid DNA, as well as kits for DNA extraction.

PCR Reagents

PCR Reagents

NIPPON GENE manufactures and supplies various PCR enzymes, including Taq DNA polymerase, which is most commonly used for PCR. NIPPON GENE also have a broad product line of dNTPs, the substrates for DNA polymerases, as well as the relevant buffers. If you need large-volume packaging and quantity purchases. Please feel free to contact us.

➢ PCR enzyme selection guide

		5'→3' Exonuclease activity	3'→5' Exonuclease Activity	Premix reagent	Ultrapure	High-speed reaction	Long chain amplification	3' end	Examples of use
Standard PCR enzymes	Especially high yield of DNA fragments of ≤1 kbp **Gene *Taq***	–	–					With dA	・Normal PCR ・TA cloning ・Low fragment amplification
	Native Taq DNA Polymerase **Gene *Taq* NT**	+	–					With dA	・Normal PCR ・TA cloning
	Minimal contamination by DNA from the host organism **Gene *Taq* FP**	–	–		●			With dA	・Normal PCR ・TA cloning ・Low fragment amplification ・RAPD PCR
	2 × premix type **Gene RED PCR Mix Plus**	+	–	●		● 10 s/kb	● ~10 kb	With dA	・Colony PCR ・Insert confirmation ・TA cloning
Hot start PCR enzymes	2 × premix type **Hot-Start Gene RED PCR Mix**	+	–	●		● 10 s/kb	● ~10 kb	With dA	・Colony PCR ・Insert confirmation ・TA cloning
	High specificity **Hot-Start Gene *Taq* NT**	+	–				● ~10 kb	With dA	・Hot-Start PCR ・Multiplex PCR ・TA cloning
	Best for multiplex PCR **Hot-Start Gene *Taq***	–	–		●			With dA	・Hot-Start PCR ・Multiplex PCR ・TA cloning
High-Fidelity enzymes	High-Fidelity, high-speed PCR **Go-to DNA Polymerase**	–	+			● 15 s/kb	● ~15 kb (*2)	Blunt	・Blunt end cloning (*1)
	High-Fidelity enzyme **Pho DNA Polymerase**	–	+				● ~20 kb	Blunt	・Long PCR ・GC rich template cloning (*1)

(*1) To use the PCR product of a highly-fidelity enzyme for TA cloning, purify the PCR product to remove the enzyme, and then perform a dA addition using the dA-overhang reaction Mix (Product Number. 313-08781).
(*2) Amplification of fragments up to 15 kb can be confirmed using λDNA as a template (at least 2.5 ng of template) with an elongation time of 15 sec/kb.

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
Standard PCR enzymes				
NIPPON GENE	312-02874	Gene *Taq*	50 U	Keep at -20 degrees C.
	318-02871		250 U	
	314-02873		250 U ×4	
NIPPON GENE	312-03234	Gene *Taq* NT	50 U	Keep at -20 degrees C.
	318-03231		250 U	
	314-03233		250 U ×4	
NIPPON GENE	315-07761	Gene RED PCR Mix Plus	48Tests	Keep at -20 degrees C.
	311-07763		96Tests	
	319-07764		960Tests	
Hot start PCR enzymes				
NIPPON GENE	315-08383	Hot-Start Gene RED PCR Mix	96Tests	Keep at -20 degrees C.
	313-08384		960Tests	
NIPPON GENE	319-07041	Hot-Start Gene *Taq*	250 U	Keep at -20 degrees C.
	315-07043		250 U ×4	
NIPPON GENE	311-07523	Hot-Start Gene *Taq* NT	250 U	Keep at -20 degrees C.
	319-07524		250 U ×4	
High-Fidelity enzymes				
NIPPON GENE	313-08661	Go-to DNA Polymerase	125 U	Keep at -20 degrees C.
	319-08663		500 U	
NIPPON GENE	314-07111	Pho DNA Polymerase	250 U	Keep at -20 degrees C.

dNTPs Mixture (25 mM each)

dNTPs Mixture (25 mM each) is a mixture of deoxynucleoside triphosphates (dNTPs), each at a concentration of 25 mM. It can be used as a substrate for DNA polymerases.

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	312-07271	dNTPs Mixture (25 mM each)	400 µL	Keep at -20 degrees C.

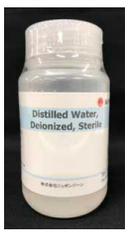
Water and Buffer Solutions

NIPPON GENE supplies a wide selection of ready-to-use, nuclease-free buffer products.

➢ Deionized Distilled Water

This product is water that has been processed by distillation, ion-exchange, and filtration, followed by autoclaving. It can be used for a wide range of applications, including the dissolution of DNA and RNA samples, as DNase- and RNase-free water. For convenience and ease of use, we also provide products that are supplied as 1 mL aliquots. Because the water is supplied in pre-dispensed aliquots, the risk of contamination during handling is minimized.

d.d. Water
100 mL



Water, Nuclease free
1 mL ×100
(314-09291)

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	316-90101	Distilled Water, Deionized	100 mL	Keep at RT.
	312-90103		100 mL ×6	
	318-90105		500 mL	
NIPPON GENE	314-09291	Water, Nuclease free	1 mL ×100	Keep at RT.

➢ DEPC treated Water

DEPC treated Water is prepared by adding diethyl dicarbonate (diethyl pyrocarbonate, DEPC) to water that has been purified by distillation, ion exchange, and filtration. The solution is then autoclaved.

In RNA-related experiments, DEPC treatment is often performed to render reagents and laboratory equipment RNase-free. To minimize the time required for such reagent preparation, we supply water that has already been treated with DEPC and autoclaved.

100 mL

500 mL

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	312-90201	DEPC treated Water	100 mL	Keep at RT.
	318-90203		100 mL ×6	
	314-90205		500 mL	

➢ TE (pH 8.0)

TE (pH 8.0) is prepared from Tris buffer (pH 8.0) and EDTA (pH 8.0), followed by autoclaving.
As a 1x TE buffer, it is ready for use. All products are nuclease-free.

100 mL

500 mL

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	314-90021	TE (pH 8.0)	100 mL	Keep at RT.
	310-90023		100 mL ×6	
	316-90025		500 mL	
NIPPON GENE	317-09281	TE (pH 8.0), Nuclease free	1 mL ×100	Keep at RT.

Cloning is a technique in which recombinant DNA is inserted into a host cell and the amount of target gene is increased to obtain many DNA fragments. A variety of processes are required for gene cloning, including insertion of DNA fragments into a cloning vector and amplification using host cells. Fujifilm Wako offers reagents necessary for a series of processes of cloning.

Cloning Vectors

The cloning vector pTS1 DNA carries T3 and T7 promoters flanking the multiple cloning site (MCS), enabling its use as a template for *in vitro* transcription with either T3 or T7 RNA polymerase.

pTS1 is a pUC-derived, high-copy-number plasmid that contains an ampicillin resistance gene. A multiple cloning site (MCS) has been inserted within the *E. coli lacZ'α* gene encoding the β-galactosidase α-peptide . When used with appropriate *E. coli* host strains (e.g., JM109), recombinant clones can be identified by blue/white screening based on *lacZ* α-complementation.

Features

- Derived from pUC-series vectors, resulting in a high copy number
- The MCS is symmetrically arranged around the Hinc II site and includes 5' overhang, blunt-end, and 3' overhang restriction enzyme sites
- The distance from the transcription start site to the Hinc II cleavage site is 55 bases for the T3 promoter and 56 bases for the T7 promoter
- Suitable for analysis by cycle sequencing using two types of primers
- The MCS is inserted into the *E. coli lacZ'α* gene fragment encoding the β-galactosidase α-peptide , enabling blue/white screening of transformed *E. coli* colonies

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	300-10123	Cloning Vector pTS1 DNA	10 µg	Keep at -20 degrees C.

Plating Beads

Bac'n'Roll Beads are plating beads used to spread *E. coli* competent cells onto LB agar plates and similar media. Approximately 10 beads are used per plate, allowing up to about 100 platings in total. The bead surface is treated with a special coating, which enables more consistent recovery of a higher number of colonies compared with uncoated beads or conventional glass spreaders.

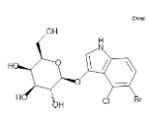
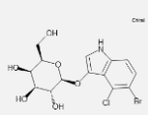
Features

- Enables consistent recovery of a larger number of colonies
- Allows simultaneous plating of multiple plates
- Can be reused up to approximately 10 times after washing and autoclaving
- No need for flame sterilization for each use, unlike spreaders

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	314-06251	Bac'n' Roll Beads	100Tests	Keep at RT.

Blue/White Screening Reagents

Used in experimental systems that employ the *lac* operon as a screening marker.

Manufacturer	Product No.	Product Name	Structure	Grade / CAS RN®	Pkg. Size	Storage Condition
FUJIFILM Wako Pure Chemical	023-15041	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside		for Molecular Biology	100 mg	Keep at 2-10 degrees C.
	029-15043			7240-90-6	1 g	
FUJIFILM Wako Pure Chemical	029-07853	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)		for Biochemistry	100 mg	Keep at 2-10 degrees C.
	027-07854				1 g	
	021-07852			7240-90-6	5 g	

Ligation Cloning Kit

➢ Ligation-Convenience Kit

The Ligation-Convenience Kit is a 2x ligation mix that enables rapid and simple DNA ligation. This product contains all components required for DNA ligation, including reaction buffer, ATP, DTT, and T4 DNA Ligase. DNA ligation can be performed simply by adding an equal volume of the 2x ligation mix to the DNA solution.

Features

- High-efficiency DNA ligation achievable in a short time (5–30 min at 16 °C)
- Simply mix an equal volume of the 2x ligation mix with the DNA solution
- No optimization required for different DNA end types
- The completed reaction mixture can be used directly for transformation
- Standard reaction volume: 20 µL
- No reduction in ligation efficiency after repeated freeze–thaw cycles (up to 50 cycles)

Applications

- **Performance Comparison in Blunt-End Ligation**

The Ligation-Convenience Kit, Product A, and Product B were compared in blunt-end ligation reactions—which are generally regarded as having low ligation efficiency—by evaluating the white colony ratio (ligation efficiency) and total number of colonies (reflecting both ligation and transformation efficiency).

(Methods)

1. pBluescript II SK(+) was digested with EcoRV, dephosphorylated, deproteinized, and dissolved in TE buffer.

2. A 500-bp DNA fragment derived from λDNA was digested with EcoRV.

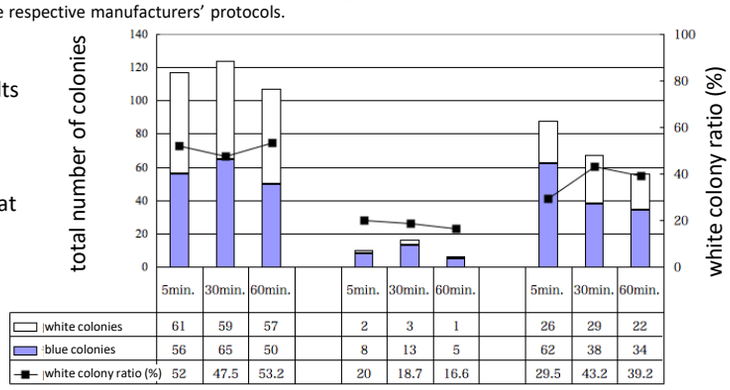
3. A 10 µL DNA solution was prepared containing 50 ng of pBluescript II SK(+) and 20 ng of the insert DNA fragment (insert/vector molar ratio = 1:2.4).

4. To the 10 µL DNA solution, 10 µL of 2x ligation mix was added and mixed thoroughly, followed by incubation at 16°C for 5–60 minutes. After the reaction, 5 µL of the reaction mixture was used to transform 50 µL of JM109 competent cells, and the resulting number of colonies were counted. For the other kits, reactions were performed in accordance with the respective manufacturers' protocols.

(Results)

With the Ligation-Convenience Kit, favorable results were obtained for both the white colony ratio (ligation efficiency) and the total number of colonies (ligation efficiency and transformation efficiency), even in blunt-end ligation reactions that are generally considered inefficient.

In addition, a reaction time of 5 minutes was sufficient.



	5min.	30min.	60min.	5min.	30min.	60min.	5min.	30min.	60min.
white colonies	61	59	57	2	3	1	26	29	22
blue colonies	56	65	50	8	13	5	62	38	34
white colony ratio (%)	52	47.5	53.2	20	18.7	16.6	29.5	43.2	39.2

Ligation-Convenience Kit Product A Product B

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	315-05963	Ligation-Convenience Kit	10Tests	Keep at -20 degrees C.
	319-05961		100Tests	

➢ dA-overhang reaction Mix

This reagent is designed for the addition of dA to 3’ termini. High-fidelity PCR enzymes of the α type possess 3’→5’ exonuclease (proofreading) activity, resulting in PCR products with blunt ends. For TA cloning of such PCR products, the addition of a dA residue to the 3’ ends is required.
This product is a 10x reaction solution that enables the simple addition of dA to blunt-ended PCR products. The dA-tailed PCR products treated with this product can be used directly for TA cloning.

Features

- Adds dA to blunt-ended PCR products
- Reaction completed in only 10 minutes at 65 °C
- dA-tailed PCR products can be used directly for TA cloning

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	313-08781	dA-overhang reaction Mix	25 µL	Keep at -20 degrees C.

ISOSPIN Plasmid

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ISOSPIN Plasmid is a kit that enables simple extraction of high-purity plasmid DNA from *E. coli* using a spin column.

This kit is based on the principle that DNA binds to silica in the presence of chaotropic ions and eliminates the need for toxic organic solvents such as phenol or chloroform. The spin columns are designed to maximize column volume, and the enclosed silica gel membrane provides sufficient DNA binding capacity and high elution efficiency. Plasmid DNA obtained using this kit is suitable for molecular biology applications such as restriction enzyme digestion, transformation, and sequencing.

Features

- Proprietary spin columns developed in-house by NIPPON GENE
- Does not use toxic organic solvents
- High-purity plasmid DNA can be extracted from *E. coli*
- Maximum plasmid size: 20 kbp
- Processing time: approximately 30 minutes

Kit contents

- IS1 Buffer 30 mL×1
- IS2 Buffer 30 mL×1
- IS3 Buffer 40 mL×1
- ISPW Buffer 60 mL×1
- ISW Buffer 100 mL×1
- ISE Buffer 10 mL×1
- Rnase A (100 mg/mL) 60 µL×1
- Spin Column 50×2 packs

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	318-07991	ISOSPIN Plasmid	100Tests	Keep at 2-10 degrees C.

ISOSPIN PCR Product

ISOSPIN PCR Product is a kit designed for purification of PCR products from PCR reaction mixtures using spin columns. The spin columns are designed to maximize column volume, and the enclosed silica membrane provides sufficient DNA binding capacity and high elution efficiency.

With this kit, DNA polymerase, salts, primers, dNTPs, and other contaminants can be removed from PCR reaction mixtures in only approximately 20 minutes, enabling the selective recovery of PCR products only. PCR products purified using this kit are suitable for use in a wide range of molecular biology applications.

Features

- Proprietary spin columns developed in-house by NIPPON GENE
- Enables recovery of high-concentration DNA in approximately 20 minutes
- Allows easy desalting and primer removal
- Does not use toxic organic solvents

Kit contents

- ISB Buffer 100 mL ×1
- ISW Buffer 100 mL ×1
- ISE Buffer 10 mL ×1
- Spin Column 50 columns ×2

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	315-08001	ISOSPIN PCR Product	100Tests	Keep at RT.

ISOSPIN Agarose Gel

ISOSPIN Agarose Gel is a kit designed for the extraction and purification of DNA fragments from agarose gels using a spin column.
This kit can be used not only with low-melting-point agarose but also with standard agarose and high-percentage agarose gels (up to 5%), allowing DNA recovery in approximately 30 minutes with a simple procedure. The purified DNA is suitable for downstream molecular biology applications such as restriction enzyme digestion, sequencing, and cloning.

Features

- Proprietary spin columns developed in-house by NIPPON GENE
- Compatible with high-percentage agarose gels (up to 5%)
- Does not use toxic organic solvents

Kit contents

- ISAE Buffer 75 mL ×2
- ISW Buffer 100 mL ×1
- ISE Buffer 10 mL ×1
- Spin Column 50 columns ×2

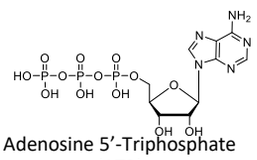
Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	311-07981	ISOSPIN Agarose Gel	100Tests	Keep at RT.

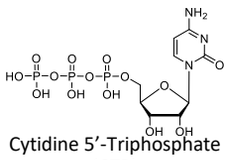
in vitro Transcription

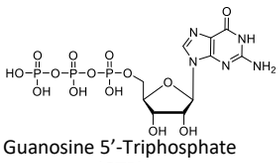
mRNA synthesis is carried out by an *in vitro* transcription (IVT) using RNA polymerase in the presence of template DNA prepared from PCR products or plasmid DNA, together with NTPs, cap analogs, and other components. FUJIFILM Wako stocks a wide range of reagents required for *in vitro* transcription.

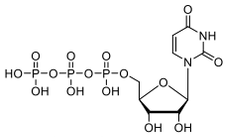
Nucleoside Triphosphate(NTPs)

Phosphorylated nucleosides serve as substrates for RNA synthesis. Natural nucleoside triphosphates include adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP). FUJIFILM Wako provides NTP solutions optimized for in vitro RNA synthesis.


Adenosine 5’-Triphosphate (ATP)


Cytidine 5’-Triphosphate (CTP)

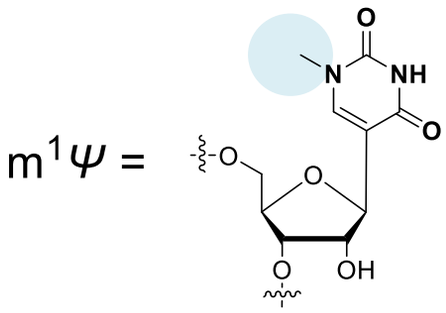

Guanosine 5’-Triphosphate (GTP)

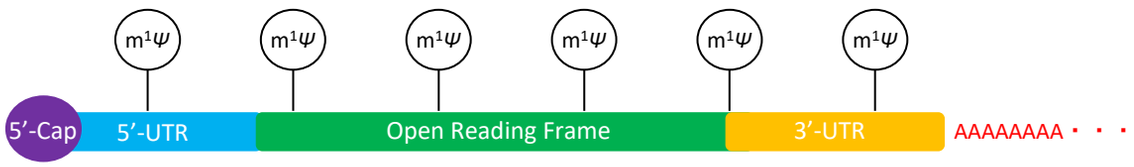

Uridine 5’-Triphosphate (UTP)

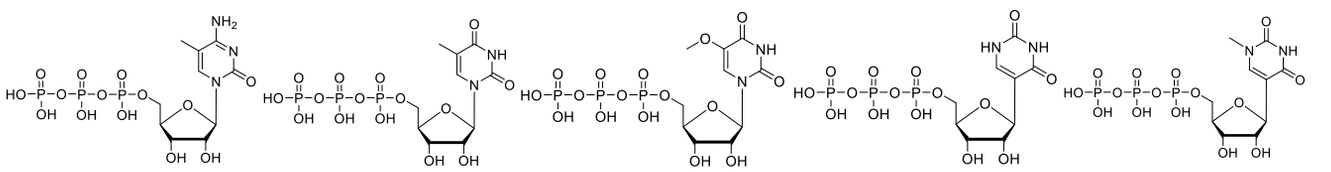
Manufacturer	Product No.	Product Name	Grade / CAS RN®	Pkg. Size	Storage Condition
ATP (Adenosine 5’-Triphosphate)					
FUJIFILM Wako Pure Chemical	012-28761	100mmol/L Adenosine 5'-Triphosphate Sodium Solution	for Nucleic Acid Synthesis	100 µL	Keep at -20 degrees C.
	018-28763		-	1 mL	
CTP (Cytidine 5’-Triphosphate)					
FUJIFILM Wako Pure Chemical	031-26251	100mmol/L Cytidine 5'-Triphosphate Sodium Solution	for Nucleic Acid Synthesis	100 µL	Keep at -20 degrees C.
	037-26253		-	1 mL	
GTP (Guanosine 5’-Triphosphate)					
FUJIFILM Wako Pure Chemical	074-06921	100mmol/L Guanosine 5'-Triphosphate Sodium Solution	for Nucleic Acid Synthesis	100 µL	Keep at -20 degrees C.
	070-06923		-	1 mL	
UTP (Uridine 5’-Triphosphate)					
FUJIFILM Wako Pure Chemical	211-01671	100mmol/L Uridine 5'-Triphosphate Sodium Solution	for Nucleic Acid Synthesis	100 µL	Keep at -20 degrees C.
	217-01673		-	1 mL	

Modified Nucleoside Triphosphates

Modified nucleoside triphosphates are nucleoside triphosphates where the nucleoside component--a key building block of DNA and RNA--has been chemically modified. One strategy to reduce the immunogenicity of mRNA therapeutics and vaccines, and to improve their stability, is to introduce chemically modified nucleosides with alterations in the base, sugar, or phosphate moieties.







5-Methylcytidine 5'-Triphosphate (5-Me-CTP) | 5-Methyluridine 5'-Triphosphate (5-Me-UTP) | 5-Methoxyuridine 5'-Triphosphate (5-OMe-UTP) | Pseudouridine 5'-Triphosphate (ΨTP) | N^1-Methylpseudouridine 5'-Triphosphate ($m^1\Psi$TP)

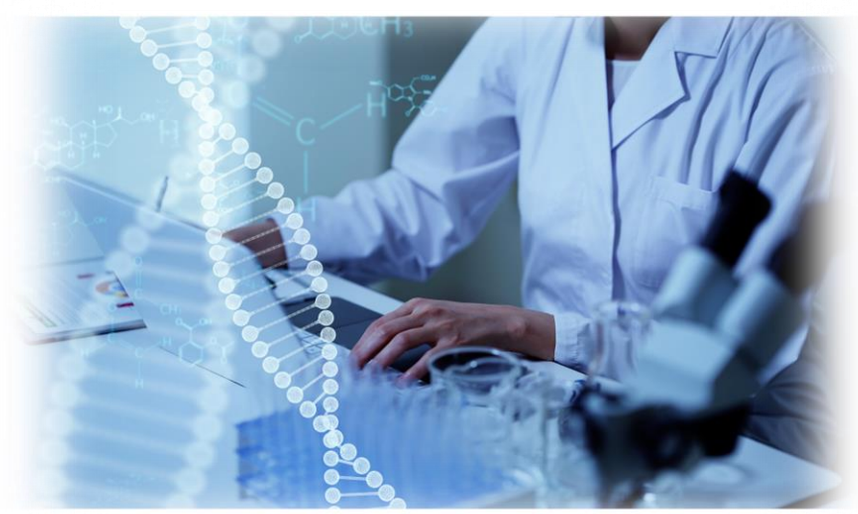
Modified Nucleoside Triphosphates

Manufacturer	Product No.	Product Name	Grade / CAS RN®	Pkg. Size	Storage Condition
CTP (Cytidine 5'-Triphosphate)					
FUJIFILM Wako Pure Chemical	130-19581	100mmol/L 5-Methylcytidine 5'-Triphosphate Sodium Solution (5-Me-CTP)	for Nucleic Acid Synthesis	100 µL	Keep at -20 degrees C.
	136-19583		-	1 mL	
UTP (Uridine 5'-Triphosphate)					
FUJIFILM Wako Pure Chemical	133-19691	100mmol/L 5-Methyluridine 5'-Triphosphate Sodium Solution (5-Me-UTP)	for Nucleic Acid Synthesis	100 µL	Keep at -20 degrees C.
	139-19693		-	1 mL	
FUJIFILM Wako Pure Chemical	137-19591	100mmol/L 5-Methoxyuridine 5'-Triphosphate Sodium Solution (5-OMe-UTP)	for Nucleic Acid Synthesis	100 µL	Keep at -20 degrees C.
	133-19593		-	1 mL	
FUJIFILM Wako Pure Chemical	165-29181	100mmol/L Pseudouridine 5'-Triphosphate Sodium Solution (ΨTP)	for Nucleic Acid Synthesis	10 µL	Keep at -20 degrees C.
	161-29183			100 µL	
	169-29184		-	1 mL	
FUJIFILM Wako Pure Chemical	135-19391	100mmol/L N^1-Methylpseudouridine 5'-Triphosphate Sodium Solution ($m^1\Psi$TP)	for Nucleic Acid Synthesis	10 µL	Keep at -20 degrees C.
	131-19393			100 µL	
	139-19394		-	1 mL	

Other Related Reagents

Related reagents used for *in vitro* transcription are listed below.

Manufacturer	Product No.	Product Name	Grade CAS RN®	Pkg. Size	Storage Condition
FUJIFILM Wako Pure Chemical	191-13831	Spermidine	for Molecular Biology	1 g	Keep at 2-10 degrees C.
	197-13833		124-20-9	5 g	
FUJIFILM Wako Pure Chemical	041-29351	Dimethyl Sulfoxide	for Molecular Biology	50 mL	Keep at RT.
	047-29353		67-68-5	100 mL	
FUJIFILM Wako Pure Chemical	132-19661	1mol/L Magnesium Acetate Solution	for Molecular Biology	100 mL	Keep at RT.
			-		
NIPPON GENE	310-90361	1 M $MgCl_2$	-	100 mL	Keep at RT.
			-		
FUJIFILM Wako Pure Chemical	044-33871	1mol/L (+/-)-Dithiothreitol(DTT) Solution	for Biochemistry	1 mL	Keep at -20 degrees C.
	040-33873		3483-12-3	1 mL×10	
FUJIFILM Wako Pure Chemical	131-14572	2-Mercaptoethanol, 99%	for Molecular Biology	25 mL	Keep at RT.
	133-14571		60-24-2	100 mL	
FUJIFILM Wako Pure Chemical	015-20995	2-Amino-2-Hydroxymethyl-1,3-propanediol Hydrochloride	for Molecular Biology	500 g	Keep at RT.
			1185-53-1		
NIPPON GENE	311-90075	0.5 M EDTA (pH 8.0)	-	500 mL	Keep at RT.
			-		



CUGA 7 *in vitro* Transcription Kit

The CUGA *in vitro* Transcription Kit is an RNA synthesis kit designed for *in vitro* transcription using CUGA RNA polymerase. The CUGA 7 RNA polymerase included in this kit exhibits higher transcription efficiency than wild-type T7 RNA polymerase, making it ideal for applications requiring large-scale RNA synthesis, such as riboprobe preparation, as well as for experiments that require the synthesis of RNA with precise chain length, including RNA structural analysis.

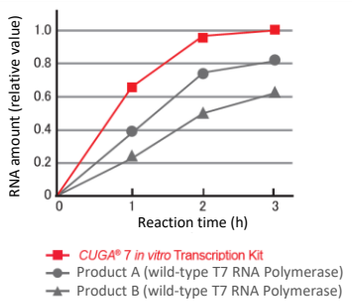
Kit contents

- CUGA 7 Enzyme Solution
- 5×Transcription Buffer
- 0.1 M DTT
- CTP, UTP, GTP, ATP (100 mM/tube each)
- Control DNA
- DNase Enzyme Solution
- 10 M Ammonium acetate
- Enzyme Dilution Buffer

Features

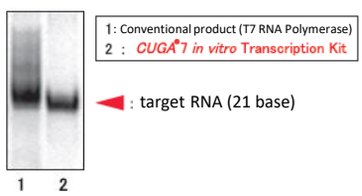
- **High-Yield RNA Synthesis**

Large amounts of RNA can be synthesized regardless of the type or length of the DNA template, including plasmid DNA, PCR products, and chemically synthesized oligonucleotides. Compared with conventional kits, approximately 2- to 5-fold higher yields can be obtained. Because CUGA polymerase and ribonucleotides are used at high concentrations, this kit is also suitable for the preparation of short-chain RNA (≤1,000 bases).



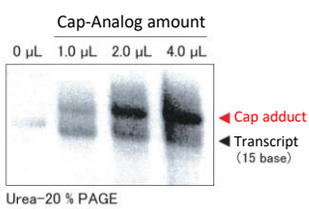
- **Consistent and Accurate Transcription**

In conventional in vitro RNA synthesis reactions, aberrant RNA can be synthesized from templates with 3′ overhang ends. In contrast, the CUGA 7 *in vitro* Transcription Kit enables accurate RNA synthesis regardless of the terminal structure of the template, resulting in higher yields of the target RNA compared with conventional products.



- **Efficient Incorporation of Cap Analogs**

Cap analog incorporation was evaluated by adding a cap analog (m7G(5′)pppG(5′)) to reactions using the CUGA 7 *in vitro* Transcription Kit, and the results confirmed that the cap analog is incorporated into the synthesized RNA.



- **Applicable to a Wide Range of Research Fields**

RNA obtained using the CUGA 7 *in vitro* Transcription Kit can be directly used in the existing experimental systems that utilize in vitro transcription reactions, such as those listed on the right. In addition, because the four ribonucleotides are supplied separately, the incorporation of modified nucleotides or cap analogs enables the construction of an even broader range of experimental systems.

Applications
- Application to cell-free protein synthesis systems
- Preparation of probes for high-sensitivity microarrays
- Preparation of probes for various hybridization analyses
- RNA structural analysis
- Preparation of functional RNAs (antisense RNA, siRNA, miRNA, etc.)
- Amplification of trace amounts of mRNA
- SELEX method

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	304-14641	CUGA 7 *in vitro* Transcription Kit	5Reactions	Keep at -20 degrees C.
	307-13531		20Reactions	Keep at -20 degrees C.

➢ Related products

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	301-15491	CUGA 3 *in vitro* Transcription Kit	5Reactions	Keep at -20 degrees C.
	307-15493		20Reactions	
NIPPON GENE	303-88221	CUGA 6 *in vitro* Transcription Kit	5Reactions	Keep at -20 degrees C.
	309-88223		20Reactions	

DNA Removal / RNA Purification Reagents

mRNA synthesized by *in vitro* transcription may contain residual template DNA and unreacted ribonucleotides. Because such contaminants can affect the quality of mRNA, it is essential to remove them through appropriate treatment. FUJIFILM Wako supplies reagents for the removal of residual DNA and for RNA purification.

DNase

DNase I is an enzyme that catalyzes the degradation of single-stranded and double-stranded DNA, producing mono- or oligonucleotides containing a 5'-phosphate group.

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	317-09661	DNase Ⅰ(RNase free)	2,000 units	Keep at -20 degrees C.
NIPPON GENE	311-08081	Deoxyribonuclease Glycerol Solution	20,000 units	Keep at -20 degrees C.

RNase inhibitor : *E. coli* expression

When handling RNA in your experiments, considerable care must be taken to avoid contamination with an RNA-degrading enzyme (RNase). RNase is considered to be a very stable enzyme but can be inactivated effectively, using specific reagents. An RNase inhibitor derived from rat lung (NIPPON GENE) is also available.

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
FUJIFILM Wako Pure Chemical	181-01821	Ribonuclease Inhibitor, Human Placenta, recombinant, Solution	5,000 units	Keep at -20 degrees C.
	187-01823		25,000 units	
NIPPON GENE	318-09691	RNase Inhibitor (high conc.)	125 µg	Keep at -20 degrees C.

Proteinase K

Proteinase K is a serine protease that mainly breaks down peptide bonds involving the carboxyl groups of hydrophobic aliphatic and aromatic amino acids. This enzyme rapidly degrades and inactivates DNase/RNase, and is therefore used in the purification of nucleic acids. This product is for Molecular Biology Grade and has been verified to be free of DNase and RNase activity.

Manufacturer	Product No.	Product Name	Grade	Pkg. Size	Storage Condition
			CAS RN®		
FUJIFILM Wako Pure Chemical	161-28701	Proteinase K, recombinant, Solution	for Molecular Biology	5 mL	Keep at -20 degrees C
	169-28702		39450-01-6	25 mL	

Chaotropic agents

This product destroys cells as a chaotropic agent to solublize insoluble proteins. It is also used for extracting RNA from tissues or cultured cells because it inactivates RNase.As a reagent of the molecular biology grade, it has been confirmed for DNase and RNase activities.

Manufacturer	Product No.	Product Name	Grade CAS RN®	Pkg. Size	Storage Condition
FUJIFILM Wako Pure Chemical	072-05001	Guanidine Hydrochloride	for Molecular Biology 50-01-1	100 g	Keep at RT.
	074-05005			500 g	
	078-05003			1 kg	
FUJIFILM Wako Pure Chemical	073-04992	Guanidine Thiocyanate	for Molecular Biology 593-84-0	25 g	Keep at RT.
	075-04991			100 g	
	077-04995			500 g	
FUJIFILM Wako Pure Chemical	215-01211	Urea	for Molecular Biology 57-13-6	100 g	Keep at RT.
	217-01215			500 g	
	211-01213			1 kg	
FUJIFILM Wako Pure Chemical	193-14491	Sodium Iodide	for Molecular Biology 7681-82-5	100 g	Keep at RT.
	195-14495			500 g	

Ethachinmate

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"Ethachinmate" is a high–molecular-weight carrier used for the precipitation of nucleic acids (DNA and RNA) with ethanol or isopropanol. It enables the efficient recovery of trace amounts of nucleic acids. It has been tested to be DNase-free and RNase-free.

Features

- Recovery of trace nucleic acids
- Rapid alcohol precipitation
- No inhibition of enzymatic reactions
- Visible precipitation
- Tested DNase and RNase-free

Manufacturer	Product No.	Product Name	Pkg. Size	Storage Condition
NIPPON GENE	318-01793	Ethachinmate	0.02 mL	Keep at 2-10 degrees C.
	312-01791		0.2 mL	

Lithium Chloride Precipitation

Lithium chloride precipitation is commonly used to remove residual NTPs and RNA polymerase.

Manufacturer	Product No.	Product Name	Grade / CAS RN®	Pkg. Size	Storage Condition
FUJIFILM Wako Pure Chemical	121-05242	Lithium Chloride	for Molecular Biology / 7447-41-8	25 g	Keep at RT.
	123-05241			100 g	
	129-05243			500 g	
NIPPON GENE	311-90075	0.5 M EDTA (pH 8.0)	- / -	500 mL	Keep at RT.
FUJIFILM Wako Pure Chemical	059-07895	70vol% Ethanol	for Molecular Biology / 64-17-5	500 mL	Keep at RT.
FUJIFILM Wako Pure Chemical	052-07221	Ethanol (99.5)	for Molecular Biology / 64-17-5	100 mL / 500 mL	Keep at RT.
	054-07225				

Ethanol Precipitation

Ethanol precipitation is commonly used to concentrate purified RNA.

Manufacturer	Product No.	Product Name	Grade / CAS RN®	Pkg. Size	Storage Condition
FUJIFILM Wako Pure Chemical	014-20482	Ammonium Acetate	for Molecular Biology / 631-61-8	25 g	Keep at RT.
	018-20485			500 g	
	016-20481			1 kg	
FUJIFILM Wako Pure Chemical	191-13912	Sodium Acetate	for Molecular Biology / 127-09-3	25 g / 500 g	Keep at RT.
	195-13915				
FUJIFILM Wako Pure Chemical	166-21551	Potassium Acetate	for Molecular Biology / 127-08-2	100 g / 500 g	Keep at RT.
	168-21555				
FUJIFILM Wako Pure Chemical	052-07221	Ethanol(99.5)	for Molecular Biology / 64-17-5	100 mL / 500 mL	Keep at RT.
	054-07225				
FUJIFILM Wako Pure Chemical	059-07895	70vol% Ethanol	for Molecular Biology / 64-17-5	500 mL	Keep at RT.
FUJIFILM Wako Pure Chemical	166-21671	2-Propanol	for Molecular Biology / 67-63-0	100 mL / 500 mL	Keep at RT.
	168-21675				
FUJIFILM Wako Pure Chemical	019-20091	2-Amino-2-hydroxymethyl-1,3-propanediol	for Molecular Biology / 77-86-1	100 g / 500 g / 1 kg	Keep at RT.
	011-20095				
	015-20093				

Related Research Support Products

RNase Decontamination Spray/ Ethanol for Disinfection

RNase Knockout is a reagent which inactivates RNase. Use it when you are concerned about contamination of RNase such as RNA treatment experiments. The method of usage is simple. It can be inactivated by spraying it on laboratory instruments and laboratory bases and wiping it off.

Manufacturer	Product No.	Product Name	Grade	Pkg. Size	Storage Condition
FUJIFILM Wako Pure Chemical	181-03381	RNase Knockout	for Genetic Research	475 mL	Keep at RT.
FUJIFILM Wako Pure Chemical	051-09376	80vol% Ethanol	for Disinfection	500 mL (Plastic bottle)	Keep at RT.
	053-09375			500 mL	
	057-09373			5 L	
	059-09377			18 L	
FUJIFILM Wako Pure Chemical	051-09131	Ethanol Spray	for Disinfection	170 mL	Keep at RT.

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