TriLink BIOTECHNOLOGIES
part of Maravai LifeSciences

Ribonucleoside-5'-triphosphates (rNTPs)

Nucleotides, raw materials for RNA synthesis

Catalog # RUO: N-1501, N-1502, N-1503, N-1504, N-1505
Catalog # GMP: FN-1501, FN-1502, FN-1503, FN-1504

Description

Adenosine-5'-triphosphate (ATP), cytidine-5'-triphosphate (CTP), guanosine-5'-triphosphate (GTP), and uridine-5'-triphosphate (UTP) are unmodified ribonucleoside triphosphates, serving as essential building blocks of RNA molecules. Each of them consists of an unmodified base, a ribose, and a 5' triphosphate group. They can be used with modified nucleotides and TriLink's CleanCap® analogs in RNA synthesis by *in vitro* transcription.

TriLink offers both research-use-only (RUO) and good manufacturing practice (GMP) rNTPs. TriLink's GMP rNTPs are manufactured in highly controlled environments with documented procedures, traceability of materials and processes, and strict quality control measures, providing exceptional consistency, purity, and safety.

TriLink's RUO and GMP rNTPs may be ordered using the following catalog numbers:

	RUO				GMP		
	10 µmol	**25 µmol**	**100 µmol**	**bulk**	**1 mmol**	**10 mmol**	**bulk**
ATP	N-1501-10	N-1501-25	N-1501-100	N-1501-bk	FN-1501-1mmol	FN-1501-10mmol	FN-1501-bk
CTP	N-1502-10	N-1502-25	N-1502-100	N-1502-bk	FN-1502-1mmol	FN-1502-10mmol	FN-1502-bk
GTP	N-1503-10	N-1503-25	N-1503-100	N-1503-bk	FN-1503-1mmol	FN-1503-10mmol	FN-1503-bk
UTP	N-1504-10	N-1504-25	N-1504-100	N-1504-bk	FN-1504-1mmol	FN-1504-10mmol	FN-1504-bk
rNTP set	N-1505-10	N-1505-25	N-1505-100	N-1505-bk	—	—	—

They are provided 100 mM in H_2O, pH 7.5.

Use & handling

Store at or below -20°C. Upon first use, prepare aliquots to minimize freeze-thaw cycles. Use only certified RNase-free reagents and consumables with proper RNase-free technique.

QC analysis

RUO products
- pH
- Concentration: UV/Vis
- Purity: ^{31}P NMR, AX-HPLC
- Identity: ^{1}H NMR, mass spectrometry

GMP products (in addition RUO analyses)
- Characterization: appearance, residual chemicals
- Safety: endotoxin, bioburden
- Nuclease contamination: DNase and RNase detection

Product released by Quality Assurance. TriLink is certified ISO 9001:2015.

Troubleshooting

For any questions or technical support around this product, please reach out to support@trilinkbiotech.com

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Other products in this guide

CleanCap® Reagent M6, also known as CleanCap® m6AG 3' OMe, is a TriLink's patented co-transcriptional cap analog to produce mRNAs containing a naturally occurring Cap-1 structure with >95% capping efficiency. Cap-1 mRNAs show superior *in vivo* activity compared to Cap-0 mRNAs produced by legacy capping methods such as mCap or anti-reverse cap analog (ARCA).

mRNA constructs created with CleanCap® Reagent M6 may further increase protein expression relative to previous generations of cap analogs, such as CleanCap® AG or CleanCap® AG (3' OMe), or mRNAs produced by enzymatic capping strategies[1].

Modified uridines such as N1-methylpseudouridine, 5-methoxyuridine, and pseudouridine can reduce immunogenic response and enhance translational efficiency of mRNAs. These properties can result in safer mRNA and increased protein expression.

Related TriLink products

Adenosine-5'-Triphoshpate, ATP (cat no. N-1501)
Cytidine-5'-Triphosphate, CTP (cat no. N-1502)
Guanosine-5'-Triphosphate, GTP (cat no. N-1503)
Uridine-5'-Triphosphate, UTP (cat no. N-1504)
rNTP Set: ATP, GTP, CTP, UTP (cat no. N-1505)
rNTP Set: ATP, CTP, GTP, N1MePsUTP (cat no. N-1506)[*]
rNTP Set: ATP, CTP, GTP, 5moUTP (cat no. N-1507)
rNTP Set: ATP, CTP, GTP, PsUTP (cat no. N-1508)

CleanCap® Reagent M6 (cat no. N-7453)
CleanCap® Reagent AG (cat no. N-7113)
CleanCap® Reagent AG (3' OMe) (cat no. N-7413)
CleanCap® Reagent AU (cat no. N-7114)

N1-Methylpseudouridine-5'-Triphosphate (cat no. N-1081)[*]
5-Methoxyuridine-5'-Triphosphate (cat no. N-1093)
Pseudouridine-5'-Triphosphate (cat no. N-1019)

T7 RNA Polymerase (Alphazyme cat no. E057)
Inorganic Pyrophosphatase (E. coli) (Alphazyme cat no. E051)
Engineered RNase Inhibitor (Alphazyme cat no. E07)

CleanCap® M6 EGFP mRNA (N1MePsU) (cat no. L-8101)[‖]
CleanCap® M6 FLuc mRNA (N1MePsU) (cat no. L-8102)
CleanCap® M6 mCherry mRNA (N1MePsU) (cat no. L-8103)
CleanCap® M6 Cas9 mRNA (N1MePsU) (cat no. L-8106)[§]
CleanCap® M6 EPO mRNA (N1MePsU) (cat no. L-8109)

Related TriLink services

TriLink offers RUO and GMP custom mRNA production services in addition to our catalog offerings. Visit our website trilinkbiotech.com/mrna-cdmo-services or contact us at mrna-services@trilinkbiotech.com for more information.

[1]Final capping is dependent upon the CleanCap® Reagent, DNA template, and final mRNA sequence. Secondary structure due to RNA length and base composition can affect final capping efficiency, mRNA yield, and translation efficiency.

Products containing CleanCap® technology are for internal research use only. A license is required for commercial use of CleanCap® Reagent M6 and other CleanCap® products. For license restrictions and patent(s) information, refer to the Research License Agreement below.

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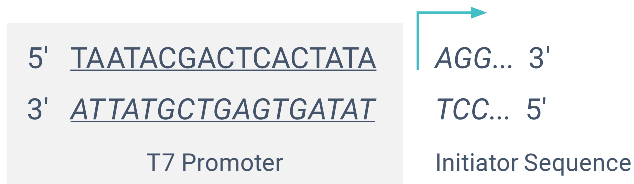
[‖]EGFP products are sold with a Limited Use Label License under sublicense with Life Technologies, Inc. Please review Limited License at trilinkbiotech.com/gfp-label-license.

RNase-free techniques

It is essential that all reagents be RNase-free. Use disposable RNase-free tubes and bottles. Surfaces and pipettes can be wiped down with RNaseZap to destroy RNases. When possible, use dedicated RNase-free pipettes. Avoid using pipettes that have been used for plasmid preparation using RNase A.

Template design

Template design is an integral part of any transcription. CleanCap M6 is to be used with the initiating sequence 5' AG 3'. The figure below shows the correct T7 promoter sequence (underlined) and initiator sequence (italics) for CleanCap M6.



AGG... 3'
TCC... 5'
Initiator Sequence

Customer supplied materials

NOTE: All reagents must be RNase-free. Use recommended source or equivalent grade.

Required reagents

- DNA template
- Nucleoside-5'-Triphosphate (NTP) Set (TriLink cat no. N-1505)
  Also available individually for use with modified NTPs. See related Products for commonly used modified NTPs.
- T7 RNA polymerase (New England BioLabs cat no. M0251S)
- Inorganic Pyrophosphatase (yeast) (New England BioLabs cat no. M2403S)
- Murine RNase Inhibitor (New England BioLabs cat no. M0314S)
- 1 M Tris-HCl (pH 7.5), RNase Free (Invitrogen cat no. 15567-027)
- Dithiothreitol (DTT) (EMD Millipore cat no. 3860-5GM)
- Spermidine (Sigma-Aldrich cat no. 85558-1G)*
- 1N HCl (Fisher Scientific cat no. SA48)
- 1 M Magnesium Chloride (Sigma-Aldrich cat no. 63069)
- UltraPure™ DNase/RNase-Free Distilled Water (Thermo Fisher Scientific cat no. 10977015)

Optional reagents

- RNaseZap™ RNase Decontamination Solution (Thermo Fisher Scientific cat no. AM9780)
- DNase I (RNase-free) (New England BioLabs cat no. M0303S)
- $CaCl_2$ (Calcium chloride solution, BioUltra, 1M) (Sigma-Aldrich cat no. 21115)
- RNeasy Kits (QIAGEN cat no. 74104 or 75144)

*NOTE: Preparation of 5 M spermidine stock solution

Undiluted spermidine stock (~6.37 M) is a viscous liquid that is difficult to pipette. To facilitate easier handling, we recommend making a 5 M dilution by adding 296 µL of nuclease-free water to a new 1 g bottle of 85558-1G and vortexing for a minimum of 60 seconds. This 5 M stock solution can then be aliquoted and stored at -20°C for at least 6 months. If using a larger starting mass than 1 g, scale the amount of water added accordingly.

10x M6 IVT transcription buffer preparation

Reagent preparation

Add reagents in the prescribed order to ensure efficient transcription and capping. Ensure each component is well-mixed and homogenous before use, which is especially important for viscous reagents like spermidine.

Table 1: 10x M6 IVT transcription buffer

Component	10x concentration	Example 1000 µL 10x M6 transcription buffer
DNase/RNase-free water	N/A	185.8 µL
Tris pH 7.5 (1M)	400 mM	400 µL
HCl (1M)	150 mM	150 µL
MgCl2 (1M)	160 mM	160 µL
DTT (1M)	100 mM	100 µL
Spermidine (5M)	21.2 mM	4.24 µL

NOTE: To enable the highest capping efficiency, this 10x M6 transcription buffer contains extra HCl outside the buffering range of Tris. Do not titrate the 10x M6 transcription buffer to achieve a target pH. Preparing this 10x M6 transcription buffer with HCl results in a low pH (<2); however, once assembled into full 1x IVT reaction with all components the pH is close to neutral (~6.8).

Standard-yield protocol

Reagent preparation

Add reagents in the prescribed order to ensure efficient transcription and capping. Ensure each component is homogenous before use. Store thawed enzymes on ice. These reaction conditions have been tested with templates up to 6 kb in length.

1. Add RNase-free water and NTPs to the reaction tube.
2. Add CleanCap M6 to the tube and vortex to mix. Spin briefly to collect liquid.
3. Add 10x M6 transcription buffer as prepared according to Table 1. Vortex. Spin briefly to collect liquid.
4. Add DNA template.
5. Add Murine RNase Inhibitor, Inorganic Pyrophosphatase, and T7 RNA Polymerase.
6. Mix well by flicking or inverting the tube 10 times and spin briefly to collect liquid.
7. Incubate at 37°C for 3 hours.

Table 2: Standard-yield reaction components

Component	Final concentration	100 µL rxn
DNase/RNase-free water	Up to 100 µL	Up to 100 µL
ATP (100 mM)	5 mM	5 µL
CTP[2] (100 mM)	5 mM	5 µL
GTP (100 mM)	5 mM	5 µL
UTP[2] (100 mM)	5 mM	5 µL
CleanCap M6 (100 mM)	10 mM	10 µL
10x M6 transcription buffer	1X	10 µL
DNA template	50 or 25 µg/mL[3]	5 µg or 2.5 µg[3]
Murine RNase Inhibitor (40 units/µL)	1 unit/µL	2.5 µL
Inorganic Pyrophosphatase (0.1 units/µL)	0.002 units/µL	2 µL
T7 RNA Polymerase (50 units/µL)	15 units/µL	30 µL
Total volume		**100 µL**

[2] Modified NTP can be used in place of wild-type. If using a modified NTP, use at the same concentration as the replaced wild-type NTP.

[3] Final concentration of DNA template should be 50 µg/mL for a plasmid template or 25 µg/mL for a PCR template.

High-yield pulse feed transcription protocol

1. Add RNase-free water and NTPs to the reaction tube.
2. Add CleanCap M6 to the tube and vortex to mix. Spin briefly to collect liquid.
3. Add 10x M6 transcription buffer as prepared according to Table 1. Vortex. Spin briefly to collect liquid.
4. Add DNA template.
5. Add murine RNase inhibitor, inorganic pyrophosphatase, and T7 RNA polymerase.
6. Mix well by flicking or inverting the tube 10 times and spin briefly to collect liquid.
7. Incubate at 37°C for 2 hours. We recommend keeping the 10x M6 transcription buffer and NTPs at 4°C after the initial reaction set up to avoid an additional freeze thaw.
8. Approximately 5 minutes before the 2-hour incubation point, prepare the Spike-In mix in a separate tube, making sure to include 10% overage to account for pipetting error.
9. Vortex to mix. Spin briefly to collect liquid.
10. Add the appropriate volume of Spike-In mix (35 μL of Spike-In per 100 μL initial volume IVT) to the original reaction tube. Pipette to mix. Cap the tube and mix well by flicking or inverting 10 times. Spin briefly to collect liquid.
11. Incubate pulse-fed reaction at 37°C for an additional 2 hours (4 hours total).

Table 3: High-yield pulse feed transcription protocol

	Component	Initial IVT mixture concentration	Volume
Initial IVT mixture	DNase/RNase-free water	Up to 100 μL	Up to 100 μL
	ATP (100 mM)	5 mM	5 μL
	CTP[2] (100 mM)	5 mM	5 μL
	GTP (100 mM)	5 mM	5 μL
	UTP[2] (100 mM)	5 mM	5 μL
	CleanCap M6 (100 mM)	10 mM	10 μL
	10x M6 transcription buffer	1X	10 μL
	DNA template	50 or 25 μg/mL[3]	5 μg or 2.5 μg[3]
	Murine RNase Inhibitor (40 units/μL)	1 unit/μL	2.5 μL
	Inorganic Pyrophosphatase (0.1 units/μL)	0.002 units/μL	2 μL
	T7 RNA Polymerase (50 units/μL)	15 units/μL	30 μL
	Total volume		**100 μL**
Incubate at 37 °C, 2 hours			
	Component	**Concentration change after pulse feed**	**Volume**
Spike-In mixture	ATP (100 mM)	Additional 4 mM	5.94 μL
	CTP[2] (100 mM)	Additional 4 mM	5.94 μL
	GTP (100 mM)	Additional 4 mM	5.94 μL
	UTP[2] (100 mM)	Additional 4 mM	5.94 μL
	10x M6 transcription buffer	Additional 0.74X[4]	14.85 μL
	Spike-In + 10% overage for 100 μL IVT		**38.61**
	Volume of Spike-In to add to 100 μL IVT		**35 μL**
	Final high-yield transcription volume		**135 μL**
Incubate at 37°C, 2 hours			

[2] Modified NTP can be used in place of wild-type. If using a modified NTP, use at the same concentration as the replaced wild-type NTP.

[3] Final concentration of DNA template should be 50 μg/mL for a plasmid template or 25 μg/mL for a PCR template

[4] The final reaction buffer will be 1.74X after addition of the Spike-In mix

Post-transcriptional options

Purifications

IVT reactions may be purified by any traditional methods such as lithium chloride precipitation or spin columns (for example, QIAGEN RNeasy mini or midi kit) for higher purity at small scales. A fixed reaction will typically result in 4-5 mg of RNA per mL of reaction using wildtype NTPs or N1-methylpseudoUTP whereas a pulse feed reaction typically produces 8-10 mg of RNA per starting mL of reaction at approximately 7.5 mg/mL concentration (crude).

DNase treatment

DNase treatment may be used per vendor-recommended protocol following first purification (above) and followed with a second small-scale RNA clean-up OR by recommended one-pot IVT/DNase reaction below.

For best one-pot DNase results formulate the final IVT reaction in 2 mM $CaCl_2$ with 20 U/µg of template DNA used in IVT at a final dilution of 4.8x the ending IVT volume by water. Mix by inverting or gently flicking the tube and pulse spin to collect liquid. Incubate for 20 minutes at 37°C.

Table 4: Example DNase reaction setup

Component	Final concentration	Fixed 100 µL IVT	Pulse-fed 135 µL IVT
IVT reaction	N/A	100 µL	135 µL
$CaCl_2$ (200 mM)	2 mM	4.8 µL	6.5 µL
DNase (2 U/µL)	20 U/µg of template DNA	25 µL[6]	25 µL[6]
Water	Variable	350.2 µL	481.5 µL
Total volume	**4.8x IVT volume**	**480 µL**	**648 µL**

[6] Volumes shown here based on IVT reaction using 25 µg/mL DNA template

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