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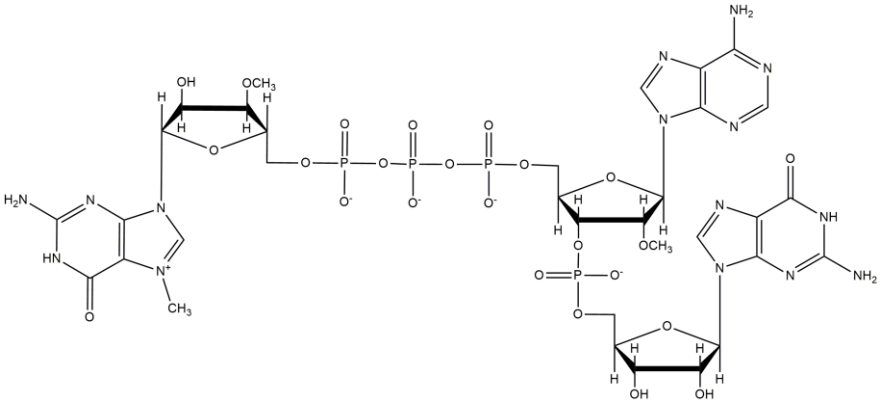
High-yield m7GmAmG mRNA synthesis kit

Catalog number: AG-mRNA-20

Description:

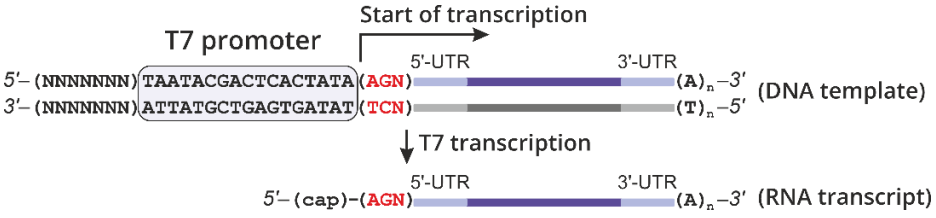
High-yield m7GmAmG mRNA synthesis kit is designed to produce Cap-1-capped mRNA via *in vitro* transcription. It is based on enzymatic synthesis of RNA transcript from DNA template using the DNA-dependent RNA polymerase of bacteriophage T7.

Molecular structure of cap analog m7GmAmG



Functional mRNA should contain the following structural features: 5' cap, 5'-UTR, correctly orientated target sequence, 3'-UTR, poly(A)-tail. Synthesized m7G-capped mRNAs possess a 100% translation efficiency. This is due to m7GmAmG is incorporated into mRNA exclusively in the correct orientation. Moreover, the transcription reaction using the trinucleotide cap analog m7GmAmG prevents the loss of RNA transcript yield that occurs when using the Anti-Reverse Cap Analog (ARCA).

The resulting mRNA can subsequently be used for a variety of applications such as mRNA structure and function studies, microinjection, transfection or *in vitro* translation experiments.



Note! Minimum T7 promoter sequence:
5'–NNNNNNNTAATACGACTCACTATAAGN...–*3'*.
First bases incorporated into RNA: AG.

Note! The use of a poly(A)-tail encoding DNA template provides for obtaining m7G-capped mRNA with poly(A)-tail in one step. Alternatively, poly(A)-tailing can posttranscriptionally be performed with poly(A) polymerase.

Content

Component	AG-mRNA-20 (20 reactions)
(×5) mRNA synthesis buffer	240 µl
(×10) DTT	120 µl
T7 RNA polymerase	70 µl
ATP	120 µl
GTP	120 µl
CTP	120 µl
UTP	120 µl
m7GmAmG	120 µl
Nuclease-free water	1 ml

(×5) T7 reaction buffer
HEPES-based buffer, salts, and other ingredients

(×10) DTT
100 mM DTT

Nuclease-free water

T7 RNA polymerase
buffered 50% (v/v) glycerol containing 300 e.a./µl T7 RNA polymerase

ATP, UTP, CTP, GTP
30 mM of each NTP

m7GmAmG
30 mM m7GmAmG

Equipment to be supplied by user

- Thermostat with the ability to keep the temperature at 37°C.
- 0.6 or 1.5 ml microcentrifuge tubes.
- Microcentrifuge.

Note! Creating a RNAse-free work environment and maintaining RNAse-free solutions is critical for performing successful *in vitro* transcription reactions. We therefore recommend using RNase inhibitor.

Companion products

- DNase (EM-100, Biolabmix).
- RNase inhibitor (RI-0020, Biolabmix).
- Pseudouridine-5'-triphosphate (TPU-0050, Biolabmix).
- 5-methylcytidine-5'-triphosphate (TMC-0050, Biolabmix).
- RNA loading buffer (D-3001, Biolabmix).
- RNA column isolation kit (RU-10, Biolabmix).

mRNA synthesis protocol

1. Reaction mix preparation

Place T7 RNA polymerase on ice. Thaw the necessary kit components, mix and pulse-spin in microfuge to collect solutions to bottom of tubes. Assemble the reaction at room temperature in the following order:

Component	Concentration	Final conc.	Volume
(×5) mRNA synthesis buffer	(×5)	(×1)	10 µl
(×10) DTT	(×10)	(×1)	5 µl
ATP	30 mM	3 mM	5 µl
UTP	30 mM	3 mM	5 µl
CTP	30 mM	3 mM	5 µl
GTP	**30 mM**	**3 mM**	**5 µl**
m7GmAmG	**30 mM**	**2.4 mM**	**4 µl**
T7 RNA polymerase	300 e.a./µl	18 e.a./µl	3 µl
DNA template	variable	variable	0.5–2 µg
Nuclease-free water			up to 50 µl
Total reaction volume			**50 µl**

2. Incubation

Mix thoroughly and pulse-spin in a microfuge. Incubate at 37°C for 2 hours.

3. DNase treatment to remove template DNA

Add 2 e.a. of DNase I, mix well and incubate at 37°C for 15 minutes. DNase treatment is optional if the template does not interfere with downstream experiment.

Note! The protocol is optimized for 0.5–2 µg DNA template, a final NTP concentration of 3 mM, m7GmAmG:GTP ratio of 4:5.

Note! A 50 µl reaction yields about 30–60 µg m7G-capped mRNA after 2 hours incubation (1 µg DNA template, 1.2 kb RNA transcript). Yields may vary depending on the template (promotor design, sequence length, secondary structure formation).

Individual optimization of protocol design

Linearized plasmid DNA or PCR products containing a double-stranded G-initiating T7 promotor region upstream of the target sequence can be used as template for *in vitro* transcription. Linearized plasmid DNA needs to be fully digested. We recommend to purify the DNA template for *in vitro* transcription.

Depending on the mRNA sequence and final application, individual reaction optimization may improve product yield and biological function (e.g. variation of incubation time, variation of DNA template amount, introduction of modified nucleotides such as m5CTP or ΨTP ratio).

For reactions with transcripts longer than 0.5 kb, 2 hours incubation should give you the maximum yield. For reactions with short RNA transcripts (< 0.5 kb), incubation time of 4 hours or longer is necessary to achieve good yield. It is safe to incubate the reaction for 16 hours (overnight).

RNA analysis

mRNA can be analyzed by gel electrophoresis in 1–2,5% agarose gel.

mRNA can be purified by LiCl precipitation, phenol:chloroform extraction followed by ethanol precipitation, or by using a spin column based method.

The amount of the purified mRNA can be estimated using UV spectrometry. The maximum of absorption for RNA corresponds to $\lambda = 260\ nm$. RNA concentration (µg/ml) can be calculated using the following formula: $A_{260} \times dilution \times 40\ \mu g/ml$. Typical optical density ratios are $A_{260}/A_{280} \geq 1.8\text{-}2.0$, $A_{260}/_{230} \geq 1.9$.

Storage

All kit components should be stored at –20 °C for up to 12 months.