T7 mRNA Transcription Kit (Cap-1)

Item No. 702880

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Customer Service 800.364.9897
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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
401018	Transcription Buffer (10X)	1 vial/50 µl	-20°C
401019	T7 RNA Polymerase Mix	1 vial/50 µl	-20°C
401021	ATP (100 mM)	1 vial/50 µl	-20°C
401023	CTP (100 mM)	1 vial/50 µl	-20°C
401024	GTP (100 mM)	1 vial/50 µl	-20°C
401025	UTP (100 mM)	1 vial/50 µl	-20°C
401026	Cap-1 (100 mM)	1 vial/40 µl	-20°C
401027	Control DNA Template	1 vial/5 µl	-20°C
401028	Nuclease-Free H_20	1 vial/500 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before using this kit.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's T7 mRNA Transcription Kit (Cap-1). This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

E-Mail: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. DNA template
2. A thermocycler, heat block, or water bath capable of heating to 37°C
3. Nuclease-free PCR tubes; 0.2 ml size recommended
4. RNA purification kit (optional, for clean up)
5. Materials used for **DNA Template Preparation** (see page 10)

INTRODUCTION

About This Kit

Cayman's T7 mRNA Transcription Kit (Cap-1) is optimized for the efficient synthesis of high-quality mRNA from a linear DNA template containing a T7 promoter sequence. Using T7 RNA polymerase, this kit enables the production of mRNA with high yield *in vitro*, which is suitable for a variety of downstream applications. The kit includes all essential reagents, including Cap-1, a modified 5'-cap analog. The cap facilitates efficient translation initiation, protects against exonuclease-mediated degradation, and improves the stability of the mRNA when delivered *via* lipid nanoparticles (LNPs) or other formulations. Each kit contains sufficient reagents for 25 reactions (20 µl per reaction). Each reaction can yield up to 100 µg of RNA.

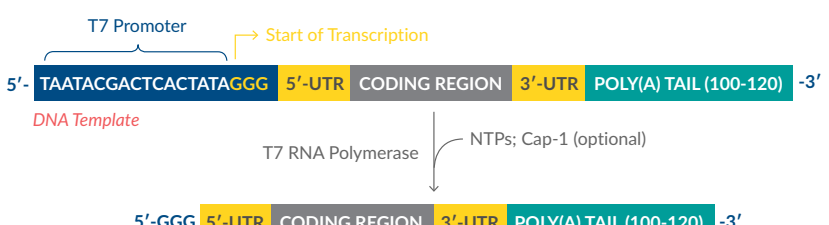

Figure 1. T7 RNA polymerase reaction scheme

PREPARATION

Reagent Preparation

Thaw all reagents on ice. Briefly centrifuge the vials at approximately 500-600 rpm for up to 5 seconds to ensure all contents are at the bottom of the vials.

1. **Transcription Buffer (10X) - (Item No. 401018)**

   This vial contains 50 µl of Transcription Buffer (10X). Once thawed, it is ready to use as supplied.

2. **T7 RNA Polymerase Mix - (Item No. 401019)**

   This vial contains 50 µl of T7 RNA Polymerase Mix. It is ready to use as supplied.

3. **ATP (100 mM) - (Item No. 401021)**

   This vial contains 50 µl of a 100 mM ATP solution. Once thawed, it is ready to use as supplied.

4. **CTP (100 mM) - (Item No. 401023)**

   This vial contains 50 µl of a 100 mM CTP solution. Once thawed, it is ready to use as supplied.

5. **GTP (100 mM) - (Item No. 401024)**

   This vial contains 50 µl of a 100 mM GTP solution. Once thawed, it is ready to use as supplied.

6. **UTP (100 mM) - (Item No. 401025)**

   This vial contains 50 µl of a 100 mM UTP solution. Once thawed, it is ready to use as supplied.

7. **Cap-1 (100 mM) - (Item No. 401026)**

   This vial contains 40 µl of 100 mM Cap-1. Once thawed, it is ready to use as supplied.

8. **Control DNA Template - (Item No. 401027)**

   This vial contains 5 µl of 0.5 µg/µl Control DNA Template. Once thawed, it is ready to use as supplied.

9. **Nuclease-Free H_2O - (Item No. 401028)**

   This vial contains 500 µl of Nuclease-Free H_2O. Once thawed, it is ready to use as supplied.

DNA Template Preparation

For optimal results, it is critical to ensure that the DNA template is high-quality and properly prepared. The success of the *in vitro* transcription reaction relies heavily on the quality, concentration, and integrity of the DNA template. The following guidelines provide important information for preparing DNA templates for use in Cayman's T7 mRNA Transcription Kit (Cap-1):

1. **DNA Template Structure**

   The DNA template should contain the following features (see Figure 1 on page 7):

   - T7 promoter sequence upstream of the coding region
   - 5′- and 3′-untranslated regions
   - Poly(A) tail, 100-120 bases in length

2. **DNA Template Preparation Methods**

   - **Plasmid DNA:** When using a plasmid DNA template, ensure that the plasmid is completely linearized prior to transcription (see Figure 2). Linearization is achieved by restriction enzyme digestion at a site downstream of the gene cassette that preferably generates a blunt end or 5'-overhang. This is important because circular plasmids can impede the transcription process. After linearization, it is recommended to purify the DNA template by phenol:chloroform extraction or by using a spin column-based purification method.

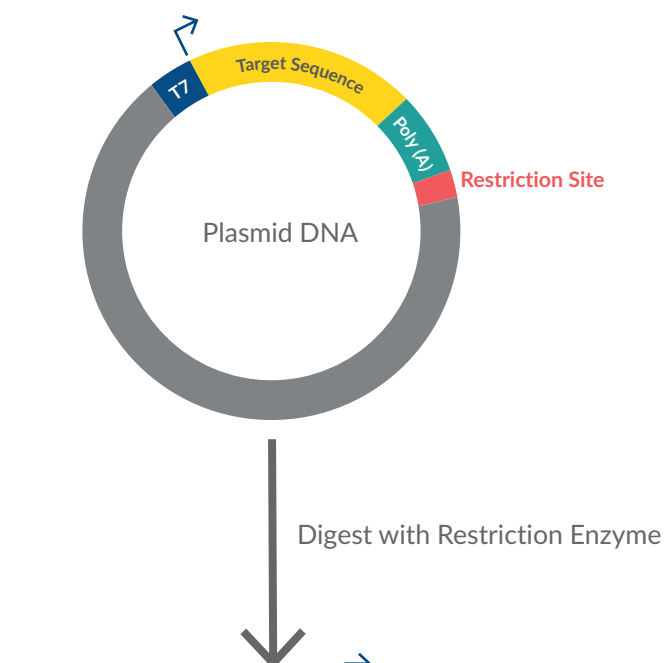

Figure 2. Linearization of plasmid DNA

- **PCR Products:** When using PCR products as a template, confirm that the product contains the T7 promoter sequence upstream of the amplified sense strand-coding region. If the T7 promoter is not included in the plasmid design, it must be added during the PCR amplification process.
    - To add the T7 promoter, design the forward PCR primer with the T7 promoter sequence (5′-TAATACGACTCACTATAGGG-3′) at the 5′ end of the coding strand. Add the sequence of the gene of interest immediately after the T7 promoter.
    - To remove any remaining enzymes and any other contaminants that may inhibit the transcription reaction, purify the template DNA using a spin column-based purification method (*i.e.*, a PCR clean-up kit).

3. **DNA Template Purity Assessment:**

   It is recommended to quantify the DNA template (*e.g.*, using a NanoDrop spectrophotometer) and ensure an A_{260}/A_{280} ratio of 1.8-2.0 for optimal purity. In addition, agarose gel or capillary electrophoresis may be used to verify the size and integrity of the template. The expected size should correspond to the DNA's predicted size, and the band should be sharp with no smearing.

4. **DNA Template Concentration:**

   The optimal concentration of DNA template for *in vitro* transcription is generally 0.5 to 2 µg per 20 µl reaction. Adjust the concentration based on the size and complexity of your template.
    - For larger DNA templates (*e.g.*, plasmids >10 kb), use 0.5 to 1 µg DNA template per 20 µl reaction.
    - For smaller DNA templates or PCR products, **use higher amounts of template DNA** (*e.g.*, 1-2 µg per 20 µl reaction).

5. **DNA Template Handling and Storage**
    - Always use nuclease-free reagents and consumables when handling the DNA template. Contamination with RNases can lead to degradation of the RNA product during or after the transcription reaction.
    - Store the prepared DNA template at or below -20°C for long-term storage.
    - If the DNA template concentration is too high, dilute it with nuclease-free water to reach the desired concentration for your transcription reaction. Avoid excessive dilution, as this may reduce the template efficiency.

PROTOCOL

Reaction Set Up

Pipetting Hints

- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the tube.

General Information

- The final volume in the reaction is 20 µl. Reactions can be scaled up as needed.
- All reagents should be prepared as described above and should be kept on ice.
- The reaction is assembled at room temperature prior to incubation at 37°C.
- Nucleases, especially RNases, can affect the transcript quality and yield. Thus, it is critical to work in a nuclease-free environment.

Performing the Transcription Reaction

1. Add the following reagents according to the order listed in the table below. Scale up as needed. **NOTE: The use of Cap-1 is optional, but highly recommended if the RNA transcript is intended for use in eukaryotic translation systems.*

	Volume (µl)	Final Concentration
Transcription Buffer (10X)	2	1X
ATP (100 mM)	2	10 mM
CTP (100 mM)	2	10 mM
GTP (100 mM)	2	10 mM
UTP (100 mM)	2	10 mM
Cap-1 (100 mM)*	1.6	8 mM
DNA Template	X	0.5-2 µg per 20 µl reaction
T7 RNA Polymerase Mix	2	--
Adjust final volume to 20 µl with Nuclease-Free H_2O		

Table 1. Pipetting summary

2. Mix thoroughly and incubate at 37°C for 2 hours. For shorter transcripts (<0.3 kb), incubate for 4 hours.
3. Purify synthesized RNA using a spin column-based clean-up kit, gel purification, or LiCl extraction and ethanol precipitation.
4. To quantify the resulting mRNA transcript, use a spectrophotometer (*e.g.*, NanoDrop). The A_{260}/A_{280} ratio should be approximately 2.0 for pure RNA.
5. To check the integrity of the mRNA transcript and/or its length, run a small aliquot of RNA on a denaturing agarose gel or capillary electrophoresis, (such as TapeStation or Bioanalyzer). Intact mRNA should show a clear, sharp band with minimal smearing on a gel. If using capillary electrophoresis look for sharp peaks with minimal degradation. Confirm the length of the transcript by comparing it to a molecular weight ladder.
6. Store RNA at -80°C.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No RNA transcript in control reaction	One or more reagents were not added to the reaction	Ensure all reagents are added to the reaction
Low yield	A. Contaminants in the DNA template are inhibiting the reaction B. The amount of DNA added is incorrect	A. Purify the DNA template B. Use the recommended amount of template in the reaction
RNA transcript size is larger than expected	DNA template plasmid was not fully linearized	Ensure that the DNA template plasmid is completely digested
Agarose gel electrophoresis of RNA transcripts appear smeary	RNase present in the DNA template	Clean-up the template using phenol:chloroform extraction

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.