Capped RNA Synthesis using a Dinucleotide Cap Analog ARCA (NEB #E2040)

Materials Required but not Supplied

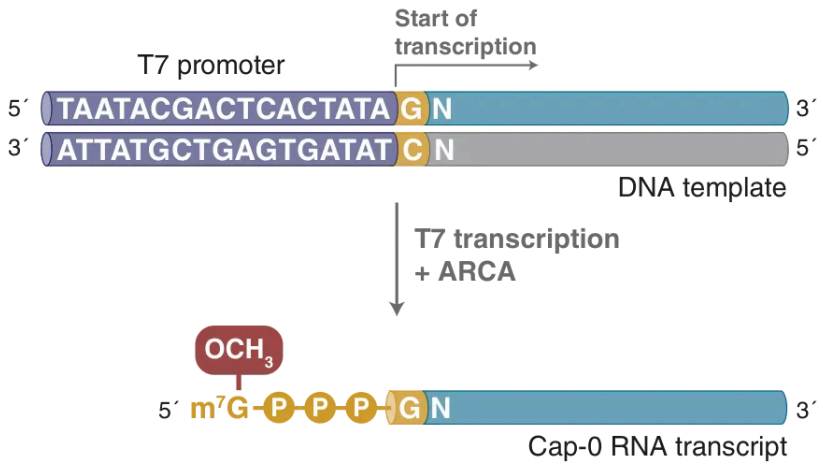
HiScribe® T7 High Yield RNA Synthesis Kit

- Nuclease-free Water (NEB #B1500)

Overview

This protocol can be used to synthesize Cap-0 RNA utilizing a dinucleotide cap analog from a DNA template containing the T7 RNA Polymerase promoter sequence immediately followed by a guanosine. Each standard 20 µl reaction yields up to 40-50 µg of RNA with approximately 80% capped RNA transcripts.

T7 RNA Polymerase co-transcriptionally caps RNA with anti-reverse cap analog (ARCA)



Before starting:

Template Considerations:

- DNA template should be purified prior to use and suspended in nuclease-free water. EDTA should not be present, and the solution should be free of salts.

- For RNA longer than 2kb, we recommend using linearized plasmid DNA as template. Completely linearized plasmid template of the highest purity is critical for successful IVT. NEB has a large selection of restriction enzymes; we recommend selecting restriction enzymes that generate blunt ends or 5’ overhangs. For very long RNA, use high-fidelity (HF) restriction enzymes to minimize star activity, if possible.

- PCR products can be used as template but we recommend using a high-fidelity DNA polymerase, such as Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493/#M0494). Though the PCR product can be directly used as template, better yields will be obtained with purified PCR products. PCR products should be examined on an agarose gel to confirm the presence of a single, robust band of the expected size. 0.1–0.5 µg of PCR product can be used in a standard 20 µl reaction.

Reaction Considerations:

- We strongly recommend wearing gloves and using nuclease-free tubes (microfuge tubes or PCR strip tubes) and reagents to avoid RNase contamination.

- For reaction times of 60 minutes or less, a water bath or heating block may be used. For reaction times longer than 60 minutes, we recommend using a dry air incubator or a thermocycler to prevent evaporation.

- The recommended ratio of dinucleotide cap analog to GTP is 4:1. Cap analogs are sold separately. See Table 1 below; increasing the ratio of cap analog to GTP will increase the proportion of capped RNA transcripts, however it also significantly decreases the yield of the transcription reaction.

Table 1. Effect of cap analog: GTP ratios on RNA yield

Dinucleotide Cap Analog: GTP Ratio	Concentration of Dinucleotide Cap Analog: GTP (mM)	RNA Yield (µg) in 2 hours	Percent Capped RNA
0:1	0:10	180	0
1:1	5:5	90-120	50%
2:1	6.7:3.3	60-90	67%
4:1	8:2	40-50	80%
8:1	8.9:1.1	20-25	89%

Protocol

1. Thaw the necessary components at room temperature. Keep the T7 RNA Polymerase Mix on ice.

2. Mix and pulse-spin in a microfuge to collect the solutions to the bottom of the tubes.

3. Prepare a 20 mM GTP solution by combining 2 µl of 100 mM GTP and 8 µl of nuclease-free water. Extra 20 mM GTP can be stored at -20°C for future use.

4. Prepare a 40 mM solution of dinucleotide cap analog.

5. Set up the reaction at **room temperature** in the order listed in the table below:

COMPONENTS	20 µl REACTION	FINAL AMOUNT
Nuclease-free water	X µl	
10X T7 Reaction Buffer	2 µl	1X
100 mM ATP	2 µl	10 mM

COMPONENTS	20 µl REACTION	FINAL AMOUNT
100 mM UTP	2 µl	10 mM
100 mM CTP	2 µl	10 mM
20 mM GTP	2 µl	2 mM
40 mM Dinucleotide Cap Analog*	4 µl	8 mM
Linear Template DNA	X µl	1 µg
100 mM DTT	1 µl	5 mM
T7 RNA Polymerase Mix	2 µl	

**Companion dinucleotide cap analog products include 3´-O-Me-m7G(5')ppp(5')G RNA Cap Structure Analog (NEB #S1411), m7G(5')ppp(5')A RNA Cap Structure Analog (NEB #S1405), G(5')ppp(5')A RNA Cap Structure Analog (NEB #S1406) or G(5')ppp(5')G RNA Cap Structure Analog (NEB #S1407).*

6. Mix thoroughly by pipetting and pulse-spin in a microfuge. Incubate at 37°C for 2 hours in a dry air incubator or thermocycler to prevent evaporation.

   Optional: To remove template DNA, add 30 µl nuclease-free water and 2 µl of DNase I (RNase-free) (NEB #M0303), mix, and incubate for 15 minutes at 37°C. Alternatively, 2 µl of DNase I-XT (NEB #M0570) can be added directly to the IVT product and incubated for 15 minutes at 37°C.

7. Proceed with purification of synthesized RNA and/or evaluation of transcription product yield and/or length. For purification, we recommend the Monarch RNA Spin Cleanup Kit with 50 µg (NEB #T2040) or 500 µg capacity (NEB #T2050).

   Optional: If a poly(A) tail is desired and is not encoded in the template plasmid, you can add one post-transcriptionally with *E. coli* Poly(A) Polymerase (NEB #M0276) using this protocol.

Related Resources

- Avoiding Ribonuclease Contamination
- Minding your caps and Poly A tails – Strategies for synthesizing *in vitro* transcribed (IVT) mRNA
- RNA Cap Analog Selection Chart
- Scaling of High-Yield *In vitro* Transcription Reactions for Linear Increase of RNA Production
- A Practical Guide to Analyzing Nucleic Acid Concentration and Purity with Microvolume Spectrophotometers