Capped RNA Synthesis (E2050)

New England Biolabs

Abstract

The kit formulation allows for efficient capped RNA synthesis using cap analog (ARCA).

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Guidelines

We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination. Reactions are typically 20 µl but can be scaled up as needed. Reactions should be assembled in nuclease-free microfuge tubes or PCR strip tubes.

The kit formulation allows for efficient capped RNA synthesis using cap analog (ARCA). The recommended ratio of cap analog to GTP is 4:1. 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 reaction. Cap analogs are sold separately. Please refer to the companion products section.

Cap analog (ARCA, NEB #S1411) is supplied in a lyophilized form of 1 µmol per tube. Dissolving it in 25 µl nuclease-free water will yield a concentration of 40 mM.

The yield per reaction is 30–40 µg RNA with approximately 80% capped RNA transcripts. Figure 1 shows the time course of capped RNA synthesis from 1 µg control template. Most reactions will be complete in 1 hour.

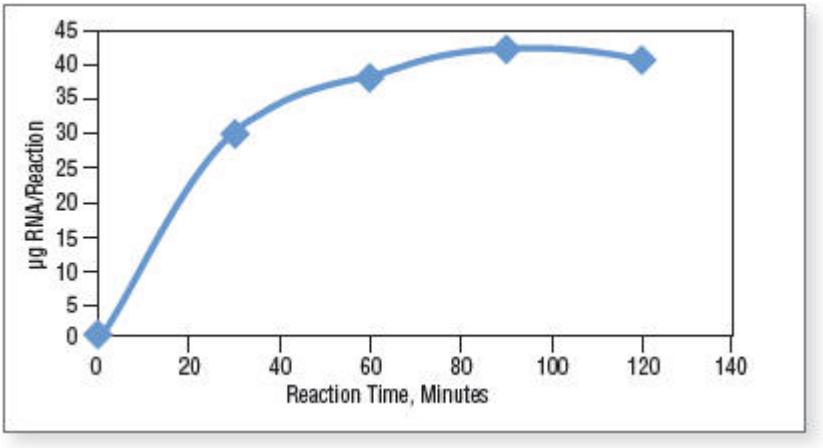

Figure 1. Capped RNA Synthesis with ARCA
Reactions were incubated at 37°C in a thermocycler. Transcripts were purified by spin columns and quantified on a NanoDrop Spectrophotometer.

Before start

We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination. Reactions are typically 20 µl but can be scaled up as needed. Reactions should be assembled in nuclease-free microfuge tubes or PCR strip tubes.

Materials

HiScribe T7 Quick High Yield RNA Synthesis Kit - 50 rxns E2050S by New England Biolabs

Protocol

Step 1.
Prepare 40 mM cap analog

REAGENTS

3'-O-Me-m7G(5')ppp(5')G RNA Cap Structure Analog - 1 umol S1411S by New England Biolabs

NOTES

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Cap analog (ARCA, NEB #S1411) is supplied in a lyophilized form of 1 µmol per tube. Dissolving it in 25 µl nuclease-free water will yield a concentration of 40 mM.

Step 2.
Thaw the necessary kit components, mix and pulse-spin in a microfuge to collect solutions to the bottoms of tubes

Step 3.
Assemble the reaction at room temperature in the following order:

PROTOCOL

. Capped RNA E2050 Mixture

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Step 3.1.
Nuclease-free water so that total volume is **20 µl**

Step 3.2.
NTP Buffer Mix, **2 µl** (2 mM each NTP final)

AMOUNT
2 µl Additional info:

Step 3.3.
Cap Analog (40 mM), **4 µl** (8 mM final)

AMOUNT
4 µl Additional info:

Step 3.4.
Template DNA, X µl (1 µg)

Step 3.5.
T7 RNA Polymerase Mix, **2 µl**

AMOUNT
2 µl Additional info:

Step 4.
Mix thoroughly and pulse-spin

Step 5.
Incubate at 37°C for 2 hours

DURATION
02:00:00

NOTES
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The yield per reaction is 30–40 µg RNA with approximately 80% capped RNA transcripts. See guidelines for figure showing the time course of capped RNA synthesis from 1 µg control template.
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Most reactions will be complete in 1 hour.

Step 6.
Optional step: To remove template DNA, add **2 µl** of DNase I (RNase-free), mix and incubate at 37°C for 15 minutes.

Step 7.
Proceed with purification of synthesized RNA or analysis of transcription products by gel electrophoresis