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Feb 14, 2022

Capped RNA Synthesis (E2050) V.2

New England Biolabs[1]

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New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

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

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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.

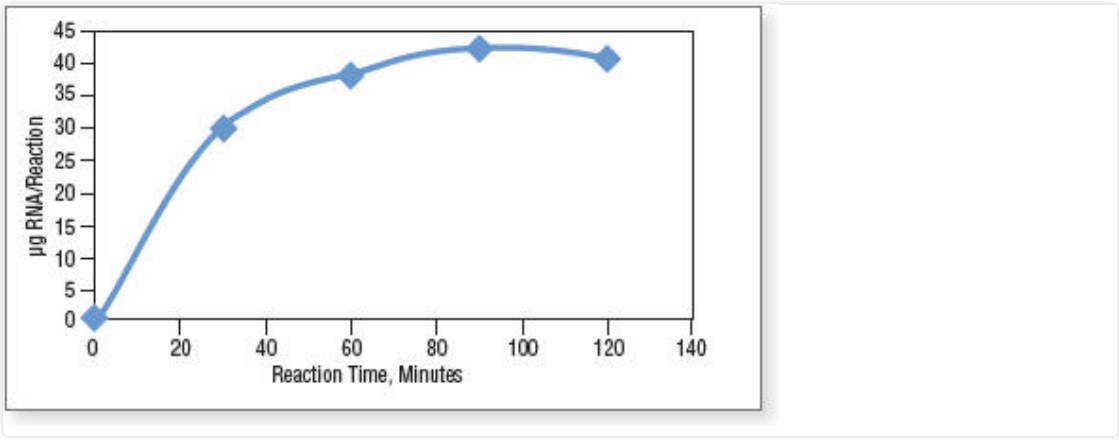

Figure 1. Capped RNA Synthesis with ARCA
Reactions were incubated at 37°C in a thermocycler. Transcripts were purified by spin columns and quantified on a NanoDrop Spectrophotometer.

MATERIALS

HiScribe T7 Quick High Yield RNA Synthesis Kit - 50 rxns **New England Biolabs Catalog #E2050S**

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

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.

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section.

1

Prepare [M]**40 Milimolar (mM) cap analog** .

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.

2

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

3

Assemble the reaction at **Room temperature** in the following order:

A	B
Reagent	**Volume**
Nuclease-free water	X µl
NTP Buffer Mix	2 µl (2 mM each NTP final)
Cap Analog (40 mM)	4 µl (8 mM final)
Template DNA	X µl (1 µg)
T7 RNA Polymerase Mix	2 µl
Total reaction volume	*20 µl*

4

Mix thoroughly and pulse-spin.

5

Incubate at **37 °C** for **02:00:00** .

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.

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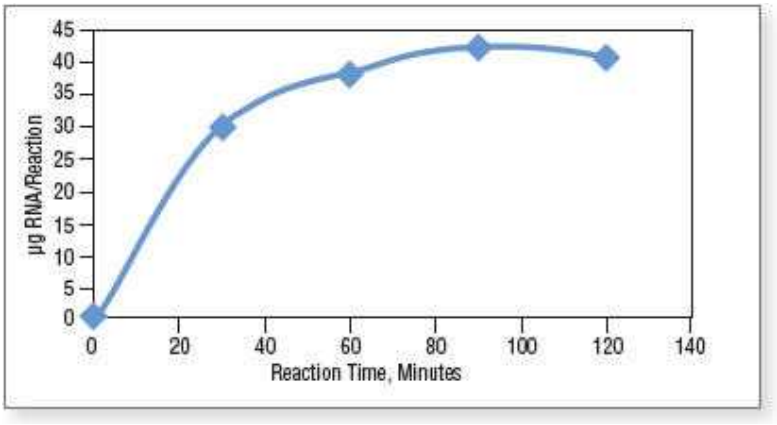

Figure 1. Capped RNA Synthesis with ARCA. Reactions were incubated at 37°C in a thermocycler. Transcripts were purified by spin columns and quantified on a NanoDrop Spectrophotometer.

6

Optional step: To remove template DNA, add **2 µL DNase I (RNase-free)** , mix and incubate at **37 °C** for **00:15:00** .

7 Proceed with purification of synthesized RNA (we recommend the Monarch RNA Cleanup Kits, NEB #T2040 or #T2050) or analysis of transcription products by gel electrophoresis.

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