2

Feb 10, 2022

RNA Synthesis with Modified Nucleotides (E2050) V.2

New England Biolabs[1]

[1]New England Biolabs

1

dx.doi.org/10.17504/protocols.io.bg54jy8w

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

New England Biolabs
New England Biolabs

This is the synthesis protocol for modified nucleotides using the HiScribe™ T7 Quick High Yield RNA Synthesis Kit (E2050). The kit is capable of synthesizing biotin- or dye-modified RNA.

DOI

dx.doi.org/10.17504/protocols.io.bg54jy8w

https://www.neb.com/protocols/2013/04/02/rna-synthesis-with-modified-nucleotides-e2050

New England Biolabs 2022. RNA Synthesis with Modified Nucleotides (E2050). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bg54jy8w New England Biolabs

synthesizing RNA with modified nucleotides, synthesizing biotin RNA, synthesizing dye-modified RNA

protocol ,

Jun 03, 2020

Feb 10, 2022

37788

Figure 1 shows the time course of labeled RNA synthesis using 1 µg control template with Biotin-16-UTP and Fluorescein-12-UTP following the above reaction setup.

Modified ribonucleotides reduce transcription efficiency; therefore, lower transcription yields should be expected as compared to transcription using unmodified NTPs. In general, Biotin-NTP and Aminoallyl-NTP have an insignificant effect on yields, while lower yields can be expected for transcription reactions containing Fluorescein-NTP or Cy-NTP. In addition, transcripts containing modified ribonucleotides have reduced electrophoretic mobility due to higher molecular weight.

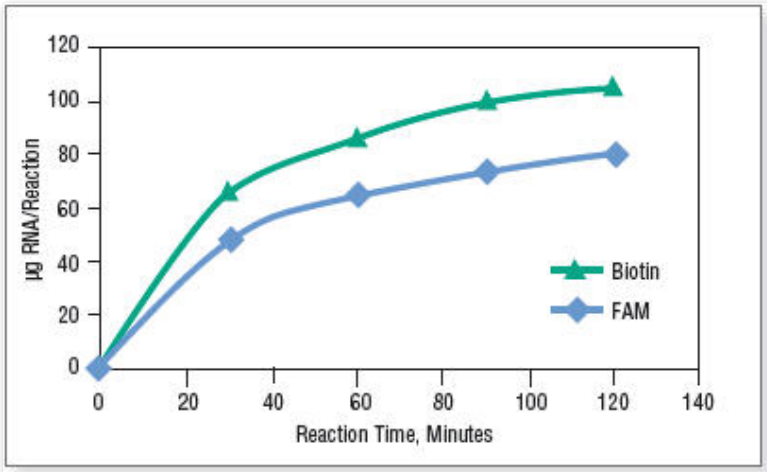


Figure 1. RNA synthesis with modified nucleotides
Reactions were incubated at 37°C in a thermocycler. Transcripts were purified by spin columns and quantified on a NanoDrop Spectrophotometer.

MATERIALS

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

HiScribe T7 High Yield RNA Synthesis Kit - 50 rxns **New England Biolabs Catalog #E2040S**

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

We strongly recommend wearing gloves.

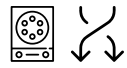
We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination. Reactions are typically **20 µL** but can be scaled up as needed. Reactions should be assembled in nuclease-free microfuge tubes or PCR strip tubes.

The kit is capable of synthesizing biotin- or dye-modified RNA with the following protocol. The recommended molar ratio of modified NTP (Biotin-, Fluorescein-, Digoxigenin-, or Aminoallyl-NTP) to standard NTP is 1:2. The following reaction set-up assumes modified UTP is used.

Citation: New England Biolabs RNA Synthesis with Modified Nucleotides (E2050) https://dx.doi.org/10.17504/protocols.io.bg54jy8w

Please note that Dye- or Biotin-NTPs are not supplied with the kit.

1 Thaw the necessary kit components.

2 

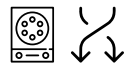
Mix and pulse-spin in microfuge to collect solutions to the bottoms of tubes.

3 

Assemble the reaction at **Room temperature** in the following order (Total reaction volume is **20 µL**):

A	B
Nuclease-free water	X µl
NTP Buffer Mix	5 µl (5 mM each NTP final)
Modified UTP (10mM)	5 µl (2.5 mM final)
Template DNA	X µl (1 µg)
T7 RNA Polymerase Mix	2 µl
Total reaction volume	20 µl

Note that the ratio of modified nucleotide to standard nucleotide can be adjusted by varying the amount of the NTP Buffer Mix and modified nucleotide. For complete modified nucleotide substitution we recommended using the T7 High Yield RNA Synthesis Kit (NEB #E2040), in which the four nucleotides are supplied separately.

4 

Mix thoroughly and pulse-spin.

5 

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

For short (< 300 nt) transcripts incubate at **37 °C** for **04:00:00** – **16:00:00** .

Citation: New England Biolabs RNA Synthesis with Modified Nucleotides (E2050) https://dx.doi.org/10.17504/protocols.io.bg54jy8w

Figure 1 shows the time course of labeled RNA synthesis using 1 µg control template with Biotin-16-UTP and Fluorescein-12-UTP following the above reaction setup.

Modified ribonucleotides reduce transcription efficiency; therefore, lower transcription yields should be expected as compared to transcription using unmodified NTPs. In general, Biotin-NTP and Aminoallyl-NTP have an insignificant effect on yields, while lower yields can be expected for transcription reactions containing Fluorescein-NTP or Cy-NTP. In addition, transcripts containing modified ribonucleotides have reduced electrophoretic mobility due to higher molecular weight.

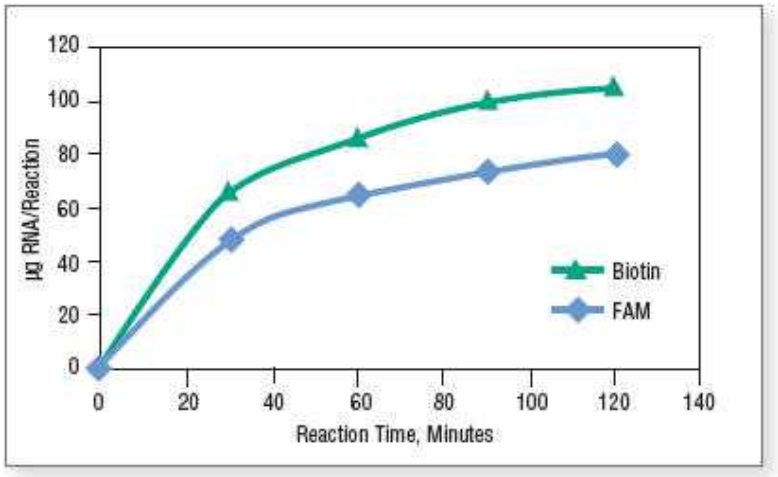


Figure 1. RNA synthesis with modified nucleotides
Reactions were incubated at 37°C in a thermocycler. Transcripts were purified by spin columns and quantified on a NanoDrop Spectrophotometer.

6

Optional step: To remove template DNA, add **30 µL nuclease-free water** and **2 µL DNase I (RNase-free)** , mix and incubate at **37 °C** for **00:15:00** .

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

Citation: New England Biolabs RNA Synthesis with Modified Nucleotides (E2050) https://dx.doi.org/10.17504/protocols.io.bg54jy8w