RNA Synthesis with Modified Nucleotides (E2050)

NEB

Abstract

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.

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

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.

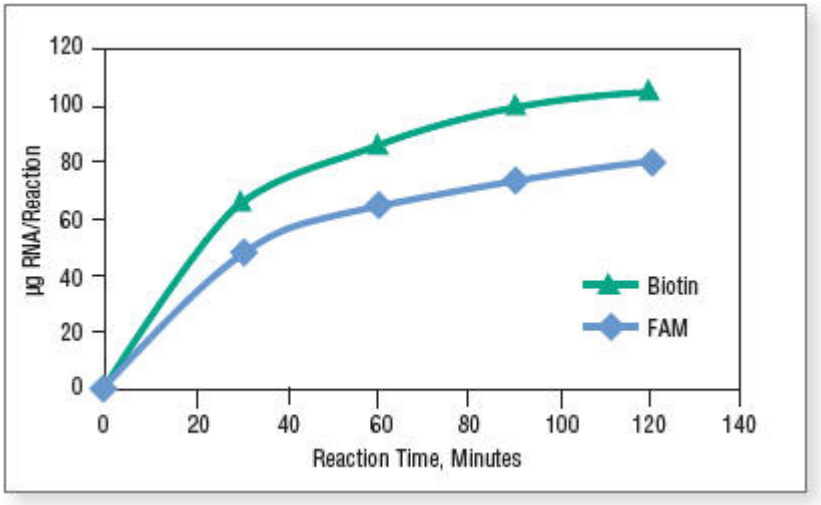

Figure 1. RNA synthesis with modified nucleotides

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 E2050S by New England Biolabs

Protocol

Step 1.
Thaw the necessary kit components

Step 2.
Mix and pulse-spin in microfuge to collect solutions to the bottoms of tubes

Step 3.
Assemble the reaction at room temperature in the following order (Total reaction volume is 20 µl):

PROTOCOL

. E2050 Modified Nucl Mixture

CONTACT: New England Biolabs

NOTES
New England Biolabs 08 Apr 2015
The recommended molar ratio of modified NTP (Biotin-, Fluorescein-, Digoxigenin-, or Aminoallyl-NTP) to standard NTP is 1:2.
New England Biolabs 08 Apr 2015
The following reaction set-up assumes modified UTP is used. (Please note that Dye- or Biotin-NTPs are not supplied with the kit.)

Step 3.1.
Nuclease-free water (X µl)

Step 3.2.
NTP Buffer Mix **5 µl** (5 mM each NTP final)

Step 3.3.
Modified UTP (10 mM): **5 µl** (2.5 mM final)

Step 3.4.
Template DNA: X µl (1 µg)

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

Step 4.
Mix thoroughly and pulse-spin

NOTES
New England Biolabs 08 Apr 2015
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.

New England Biolabs 08 Apr 2015
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.
New England Biolabs 08 Apr 2015
Mix thoroughly, pulse-spin and incubate at 37°C for 2 hours. For short (< 300 nt) transcripts incubate at 37°C for 4–16 hours.

Step 5.
Incubate at 37°C for 2 hours.

DURATION
02:00:00

Step 6.
Optional step: To remove template DNA, add 30 µl nuclease-free water and 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.