Standard RNA Synthesis (E2050)

New England Biolabs

Abstract

This is the synthesis protocol using the HiScribe™ T7 Quick High Yield RNA Synthesis Kit (E2050)

Citation: New England Biolabs Standard RNA Synthesis (E2050). **protocols.io**
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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.

Reaction time depends on template amount, quality and RNA transcript length. For reactions with transcripts longer than 0.3 kb, 2 hour incubation should give you the maximum yield. 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 of the sample.

For reactions with short RNA transcripts (< 0.3 kb), we recommend an incubation time of 4 hours or longer. It is safe to incubate the reaction for 16 hours (overnight). For example, we have achieved good yield with only 0.2 µg plasmid template encoding a 50-mer RNA by incubating the reaction overnight at 37°C.

Reaction set up for short transcripts (< 0.3 kb):

Nuclease-free water	X µl
NTP Buffer Mix	10 µl (6.7 mM each NTP final)
Template DNA	X µl (1 µg)
T7 RNA Polymerase Mix	2 µl
Total reaction volume	30 µl

Compared to the standard reaction, this reaction uses 10 µl more water. The volume of NTP Buffer Mix and T7 RNA Polymerase Mix, however, remains the same. The kit contains sufficient materials for 50 reactions.

Note that the amount of NTP Buffer Mix in a standard 20 µl reaction can vary from 2 to 10 µl. The final yield is proportional to the amount of input nucleotides, meaning that the nucleotide incorporation efficiency remains the same when different amounts of NTP are used. Figure 1 shows the time course of standard RNA synthesis from 1 µg control DNA template coding for a 1.8 kb RNA transcript with the HiScribe T7 Quick Kit using 10 µl and 5 µl NTP Buffer Mix in a 20 µl reaction.

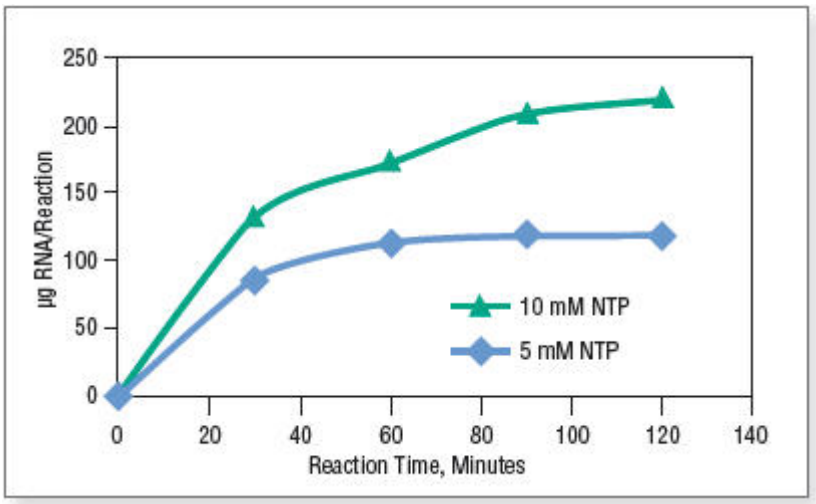

Figure 1. RNA synthesis with different amounts of NTP.
Reactions were incubated at 37°C in a thermocycler. Transcripts were purified by spin columns and quantified on a NanoDrop™ Spectrophotometer.

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.
Thaw the necessary kit components

ANNOTATIONS
Yajie Niu 06 Dec 2016

Prepare the ds oligo template by mixing equal molar of forward and reverse primer strands together at least 1µg in total

95°C 5min and then cool down to room temperature on the bench for at least 1 hr.

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

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

PROTOCOL

. Standard RNA Synthesis Mixture for E2050

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Step 3.1.
Nuclease-free water, to **20 µl** total volume

NOTES
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up to 10ul

Step 3.2.
NTP Buffer Mix, **10 µl** (10 mM each NTP final)

AMOUNT
10 µl Additional info:

NOTES
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5ul

Step 3.3.
Template DNA, X µl (1 µg)

ANNOTATIONS
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20ng/ul gblock 0.25ul

Step 3.4.
T7 RNA Polymerase Mix, **2 µl**

AMOUNT
2 µl Additional info:

NOTES
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1ul

Step 4.
Mix thoroughly and pulse-spin in a microfuge

Step 5.
Incubate at 37°C for 2 hours

DURATION
02:00:00

Step 6.
Optional step: DNase treatment to remove DNA template. To remove template DNA, add 30 µl nuclease-free water to each 20 µl reaction, followed by 2 µl of DNase I (RNase-free), mix and incubate for 15 minutes at 37°C.

NOTES
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Standard reactions are capable of generating large amounts of RNA, at concentrations up to 10 mg/ml. As a result, the reaction mixture is quite viscous. It is easier to perform DNase treatment after the reaction mixture is diluted.

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

electrophoresis.