





New England Biolabs¹

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Standard RNA Synthesis (E2050) V.2



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

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https://www.neb.com/protocols/2013/04/02/standard-rna-synthesis-e2050

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RNA Synthesis

protocol,

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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.

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.

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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 $0.2 \mu g$ plasmid template encoding a 50-mer RNA by incubating the reaction overnight at $37^{\circ}C$.

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

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

Compared to the standard reaction, this reaction uses 10 μ l 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 μ l reaction can vary from 2 to 10 μ l. 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 μ g control DNA template coding for a 1.8 kb RNA transcript with the HiScribe T7 Quick Kit using 10 μ l and 5 μ l NTP Buffer Mix in a 20 μ l reaction.

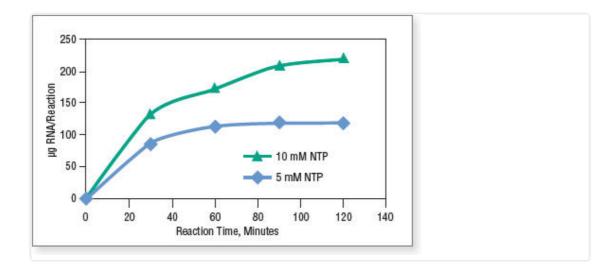


Figure 1. RNA synthesis with different amounts of NTP. Reactions were incubated at 37° C in a thermocycler. Transcripts were purified by spin columns and quantified on a NanoDrop[™] Spectrophotometer.

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

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

We strongly recommend wearing gloves.

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1 Thaw the necessary kit components.





Mix and pulse-spin in microfuge to collect solutions to the bottoms of tubes. Keep § On ice .

3

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

Α	В
Nuclease-free water	ΧμΙ
NTP Buffer Mix	10 μl (10 mM each NTP final)
Template DNA	Х µІ (1 µg)
T7 RNA Polymerase Mix	2 μΙ
Total reaction volume	20 μΙ

4

Mix thoroughly and pulse-spin in a microfuge.

5

Incubate at § 37 °C for © 02:00:00.

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 μ g plasmid template encoding a 50-mer RNA by incubating the reaction overnight at 37°C.

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

Α	В
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Total reaction volume	30 μΙ

Compared to the standard reaction, this reaction uses 10 μ l 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 μ l reaction can vary from 2 to 10 μ l. 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 μ g control DNA template coding for a 1.8 kb RNA transcript with the HiScribe T7 Quick Kit using 10 μ l and 5 μ l NTP Buffer Mix in a 20 μ l reaction.

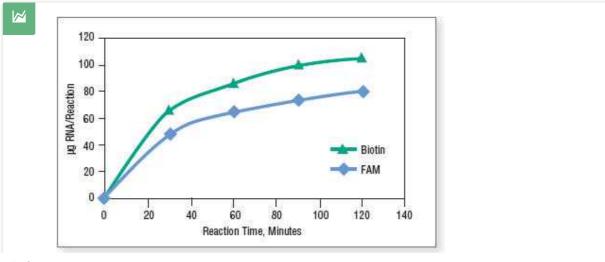


Figure 1. RNA synthesis with different amounts of NTP.

Reactions were incubated at 37°C in a thermocycler. Transcripts were purified by spin columns and quantified on a NanoDrop™ Spectrophotometer.



Optional step: To remove template DNA, add $\blacksquare 30~\mu L$ nuclease-free water to each 20 μl reaction, followed by $\blacksquare 2~\mu L$ DNase I (RNase-free), mix and incubate at $\& 37~^{\circ}C$ for @ 00:15:00.

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.

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