

May 15, 2020

Version 1

Protocol for Standard RNA Synthesis with Hi-T7 RNA Polymerase (NEB #M0658) V.1

DOI

dx.doi.org/10.17504/protocols.io.8tnhwme



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External link: <https://www.neb.com/protocols/2018/06/21/protocol-for-standard-rna-synthesis-with-hi-t7-rna-polymerase-neb-m0658>



Protocol Citation: New England Biolabs 2020. Protocol for Standard RNA Synthesis with Hi-T7 RNA Polymerase (NEB #M0658).
protocols.io <https://dx.doi.org/10.17504/protocols.io.8tnhwme>

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Protocol status: Working

We use this protocol and it's working

Created: October 28, 2019

Last Modified: May 15, 2020

Protocol Integer ID: 29262

Keywords: protocol for standard rna synthesis, t7 rna polymerase, standard rna synthesis, rna

Materials

STEP MATERIALS

☒ Nuclease-free Water **New England Biolabs Catalog #E6317**

☒ NTPs

☒ Template DNA

☒ Hi-T7 RNA Polymerase 50 U/ml **New England Biolabs Catalog #M0658S**

☒ RNase Inhibitor

☒ 10X Hi-T7 RNA Polymerase Reaction Buffer **New England Biolabs Catalog #M0658S**



Protocol materials

⊗ 10X Hi-T7 RNA Polymerase Reaction Buffer **New England Biolabs Catalog #M0658S**

⊗ Nuclease-free Water **New England Biolabs Catalog #E6317**

⊗ NTPs

⊗ Template DNA

⊗ Hi-T7 RNA Polymerase 50 U/ml **New England Biolabs Catalog #M0658S**

⊗ RNase Inhibitor

⊗ Nuclease-free Water **New England Biolabs Catalog #E6317**

⊗ 10X Hi-T7 RNA Polymerase Reaction Buffer **New England Biolabs Catalog #M0658S**

⊗ NTPs

⊗ Template DNA

⊗ RNase Inhibitor

⊗ Hi-T7 RNA Polymerase 50 U/ml **New England Biolabs Catalog #M0658S**

Troubleshooting

Safety warnings

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



Assemble the reaction

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

Assemble the reaction at Room temperature in the following order.

Note

The total reaction volume is 20 μL .

- 2 Add Nuclease-free H_2O : X μL

Nuclease-free Water **New England Biolabs Catalog #E6317**

- 3 Add 10X Hi-T7 RNA Polymerase Reaction Buffer: 2 μL [M] 1 X

10X Hi-T7 RNA Polymerase Reaction Buffer **New England Biolabs Catalog #M0658S**

Note

Hi-T7 RNA Polymerase Supplied with 10X Hi-T7 RNA Polymerase Reaction Buffer formulated for optimal performance.

- 4 Add NTPs: X μL [M] 0.5 millimolar (mM) each

NTPs

- 5 Add Template DNA: X μL from [M] 0.2 μg to [M] 1 μg

Template DNA

- 6 Add RNase Inhibitor: 0.5 μL [M] 1 U/ μL final

RNase Inhibitor



- 7 Add Hi-T7 RNA Polymerase (50 U/ μL): 2 μL





Hi-T7 RNA Polymerase 50 U/ml **New England Biolabs Catalog #M0658S**

Incubation

8 Incubate at  50 °C for  01:00:00 .

