May 16, 2020

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

New England Biolabs[1]
[1]New England Biolabs

1 Works for me 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

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Nuclease-free Water	E6317	New England Biolabs
NTPs		
Template DNA		
Hi-T7 RNA Polymerase 50 U/ml	M0658S	New England Biolabs
RNase Inhibitor		
10X Hi-T7 RNA Polymerase Reaction Buffer	M0658S	New England Biolabs

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.

The total reaction volume is **20 µl** .

2 Add Nuclease-free H_2O: X µl

Nuclease-free Water
by New England Biolabs
Catalog #: E6317

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

Citation: New England Biolabs (05/16/2020). Protocol for Standard RNA Synthesis with Hi-T7 RNA Polymerase (NEB #M0658). https://dx.doi.org/10.17504/protocols.io.8tnhwme

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

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 Milimolar (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
by New England Biolabs
Catalog #: M0658S

Incubation

8 Incubate at **50 °C** for **01:00:00** .

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