VERSION 1

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Protocol status: Working
 We use this protocol and it's working

Created: Oct 28, 2019

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

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MATERIALS

STEP MATERIALS

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

☒ NTPs **Contributed by users**

☒ Template DNA **Contributed by users**

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

☒ RNase Inhibitor **Contributed by users**

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

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 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 0.5 millimolar (mM) each

NTPs **Contributed by users**

- 5 Add Template DNA: X μl from 0.2 μg to 1 μg

Template DNA **Contributed by users**

- 6 Add RNase Inhibitor: 0.5 μL 1 U/ μl final

RNase Inhibitor **Contributed by users**

7 Add Hi-T7 RNA Polymerase (50 U/ μ l): 2 μ L

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

Incubation

8 Incubate at 50 °C for 01:00:00 .