

Protocol for Standard RNA Synthesis

Materials Required but not Supplied

T7 RNA Polymerase

- DTT
- Nuclease-free Water (NEB #B1500)
- Ribonucleotide Solution Mix (NEB #N0466)
- Ribonucleotide Solution Set (NEB #N0450)
- RNase Inhibitor, Murine (NEB #M0314) (optional)

T7 RNA Polymerase (High Concentration)

- DTT
- Nuclease-free Water (NEB #B1500)
- Ribonucleotide Solution Mix (NEB #N0466)
- Ribonucleotide Solution Set (NEB #N0450)
- RNase Inhibitor, Murine (NEB #M0314) (optional)

Overview

Assemble the reaction at room temperature in the following order.

Components	Amount	Concentration
H ₂ O	X µl	
10X Reaction Buffer	2 µl	
NTP	X µl	0.5 mM each
Template DNA	X µl	0.2–1 µg
RNase Inhibitor (optional)	0.5 µl	1 U/µl final
Fresh DTT (optional)	X µl	5 mM final
T7 RNA Pol	2 µl	
Total Volume	20 µl	

Incubate at 37°C for 1 hour. For shorter (< 300 nt) transcripts incubate at 37°C for 2–16 hours.