Removing DNA template from IVT reactions using DNase I (NEB #M0303)

Materials Required but not Supplied

DNase I (RNase-free)
- Nuclease-free Water (NEB #B1500)
- RNase Inhibitor, Murine (NEB #M0314)
- EDTA

HiScribe® T7 High Yield RNA Synthesis Kit
- Nuclease-free Water (NEB #B1500)

HiScribe® T7 Quick High Yield RNA Synthesis Kit
- Nuclease-free Water (NEB #B1500)

HiScribe® T7 ARCA mRNA Kit
- Nuclease-free Water (NEB #B1500)

HiScribe® T7 ARCA mRNA Kit (with tailing)
- Nuclease-free Water (NEB #B1500)

HiScribe® SP6 RNA Synthesis Kit
- Nuclease-free Water (NEB #B1500)

HiScribe® T7 mRNA Kit with CleanCap® Reagent AG
- Nuclease-free Water (NEB #B1500)

Overview

DNase I is commonly used for removing the DNA template in *in vitro* transcription reaction products. DNase I (RNase-free) is inhibited by monovalent salt concentrations common to IVT reactions (>50 mM), so dilution of the IVT reaction product is necessary. Additionally, high-yield IVT reactions may be viscous, and dilution helps with complete DNA template digestion. We do offer salt-tolerant DNase I-XT (NEB #M0570).

Precautions:

- Wearing gloves and using nuclease-free tubes and reagents is strongly recommended to avoid RNase contamination.

Protocol

1. Set up the reaction in the following order on ice:

COMPONENTS	52 µl REACTION	FINAL AMOUNT
Nuclease-free Water	30 µl	
IVT reaction product	20 µl	
DNase I (RNase-free)	2 µl	4 units

2. Incubate at 37°C for 15 minutes.

3. Immediately clean up the reaction using one of the methods described in the protocol for Purification of IVT RNA.

Related Resources

- GMP-grade Products for RNA Synthesis – Tools to Take you from Template to Transcript
- Scaling of High-Yield *In vitro* Transcription Reactions for Linear Increase of RNA Production