A Typical DNase I Reaction Protocol (NEB #M0303)

Materials Required but not Supplied

DNase I (RNase-free)

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

Overview

This is an example protocol for DNase I (RNase-free), which is a sequence-independent DNA endonuclease that catalyzes the cleavage of the phosphodiester bonds in ssDNA, dsDNA, chromatin, and the DNA strand of RNA:DNA hybrid duplexes to release di-, tri-, and oligonucleotide products with 5' phosphorylated and 3' hydroxylated ends.

DNase I is commonly used for removal of contaminating genomic DNA from RNA samples and degradation of the DNA template in *in vitro* transcription reactions. DNase I (RNase-free) is inhibited by monovalent salt concentrations common to IVT reactions (>50 mM); 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	100 µl REACTION	FINAL AMOUNT
Nuclease-free water	X µl	-
RNase Inhibitor, Murine *(optional)*	2.5 µl	100 units
10X DNase I Reaction Buffer	10 µl	1X
RNA	X µl	1 µg
DNase I (RNase-free)	1 µl	2 units

2. Incubate at 37°C for 10 minutes.
3. Stop the reaction by adding EDTA to a final concentration of 5 mM.
4. We recommend cleaning up the reaction with our Monarch® Spin RNA Cleanup Kits (10 µg capacity NEB #T2030, 50 µg capacity NEB #T2040, or 500 µg capacity NEB #T2050) or phenol-chloroform extraction.

If there is no RNA in your reaction, the enzyme may be heat inactivated at 75°C for 10 minutes.

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