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Protocol for removing ssDNA from dsDNA or RNA Samples (NEB #M0568) V.1



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Protocol status: Working

We use this protocol and it's working

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Abstract

The protocol described below will enable degradation of up to 20 pmol of a 25 nt ssDNA (~ 200 ng).

In order to degrade larger amounts of ssDNA or ssDNAs longer than 25 nt, we recommend adding more enzyme instead of extending the reaction time.

Users should note that ssDNAs longer than 25 nt may form secondary structures that hinder Thermolabile Exonuclease I activity.

Materials

MATERIALS

⊗ NEBuffer 3.1 - 5.0 ml **New England Biolabs Catalog #B7203S**

⊗ Thermolabile Exonuclease I **New England Biolabs Catalog #M0568**

⊗ Nuclease-free Water **New England Biolabs Catalog #B1500**

Safety warnings

⚠ Please see SDS (Safety Data Sheet) for hazards and safety warnings.



- 1 Prepare a  20 μL reaction as follows:



Sample containing ssDNA (up to 20 pmol of a 25-mer)	x μL
NEBuffer 3.1 (NEB #B7203)*	2 μL **
Thermolabile Exonuclease I	1 μL
Nuclease-free water	to 20 μL ***

*Most PCR buffers are compatible.

**No reaction buffer is necessary if enzyme is added to a PCR reaction.

***Scale larger reaction volumes proportionally.

- 2 Incubate at  37 $^{\circ}\text{C}$ for  00:04:00 .



- 3 Stop reaction by heat-inactivation at  80 $^{\circ}\text{C}$ for  00:01:00 .