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# Terminator™ 5′-Phosphate-Dependent Exonuclease

Cat. No. TER51020



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## 1. Introduction

Terminator™ 5′-Phosphate-Dependent Exonuclease* is a processive 5′→3′ exonuclease that digests RNA having a 5′ monophosphate. It does not digest RNA having a 5′-triphosphate, 5′-cap, or 5′-hydroxyl group. These enzymatic properties make Terminator Exonuclease ideal for producing mRNA-enriched samples from both eukaryotic and prokaryotic total RNA preparations by selectively digesting the ribosomal RNA (rRNA).

Terminator Exonuclease can be used to isolate eukaryotic mRNA substantially free of 18S and 28S rRNA without using an oligo(dT) matrix. Further, Terminator Exonuclease provides a simple and effective method for purifying unprocessed, primary bacterial transcripts by removing large rRNA and other processed transcripts.

Terminator Exonuclease will also digest single-stranded DNA (ssDNA) having a 5′-phosphate group. It does not digest ssDNA or dsDNA having a 5′-triphosphate or a 5′-hydroxyl group. Terminator Exonuclease is not inhibited by RNase Inhibitors such as RNasin®, Prime RNase Inhibitor™, or Epicentre's RiboGuard™ RNase Inhibitor.

Terminator 5′-Phosphate-Dependent Exonuclease is available in a 40-U size at a concentration of 1 U/µl. Two different 10X Terminator Reaction Buffers are also provided with the enzyme, each optimized for different applications.

## 2. Product Specifications

**Storage:** Store only at –20°C in a freezer without a defrost cycle.

**Storage Buffer:** Terminator Exonuclease is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.1% Triton® X-100.

**Unit Definition:** One unit digests 1 µg of rRNA substrate into acid-soluble nucleotides in 60 minutes at 30°C under standard assay conditions.

**Quality Control:** Terminator Exonuclease is function-tested in a 50-µl reaction containing 50 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, 100 mM NaCl, 20 µg rRNA, and varying amounts of enzyme.

**Contaminating Activity Assays:** Terminator Exonuclease is free of detectable contaminating endonuclease and non-5′-phosphate-dependent nuclease activities.

** Patent pending*

## 3. Related Products

The following products are also available:

- MasterPure™ RNA Purification Kit
- MasterPure™ Complete DNA and RNA Purification Kit
- ArrayPure™ Nano-scale RNA Purification Kit
- MonsterScript™ Reverse Transcriptase
- MonsterScript™ 1st-Strand cDNA Synthesis Kit
- MMLV 1st-Strand cDNA Synthesis Kit
- MMLV High Performance Reverse Transcriptase
- Tobacco Acid Pyrophosphatase
- RNA 5′ Polyphosphatase
- RiboGuard™ RNase Inhibitor

## 4. General Considerations

**Integrity of the RNA Sample:** The success of a Terminator Exonuclease reaction is strongly influenced by the quality of the total RNA sample used in the reaction. Therefore, it is important to confirm the purity and integrity of the RNA sample prior to beginning the reaction.

**RNA Sample:** The RNA sample to be treated with Terminator Exonuclease should be dissolved in RNase-free water prior to treatment.

***Important:*** *Do not dissolve the RNA sample in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA), or any other EDTA-containing buffer.*

**Maintaining an RNase-Free Environment:** Ribonuclease contamination is a significant concern for those working with RNA. The ubiquitous RNase A is a highly stable and active ribonuclease that can contaminate any lab environment and is present on human skin. Creating an RNase-free work environment and maintaining RNase-free solutions is critical for performing successful reactions. Therefore, we strongly recommend that the user:

1) Autoclave all tubes and pipette tips that will be used in the reactions.
2) Always wear gloves when handling samples containing RNA. Change gloves frequently especially after touching potential sources of RNase contamination such as door knobs, pens, pencils, and human skin.
3) Always wear gloves when handling reaction components. Do not pick up the enzyme or buffer with an ungloved hand.
4) Keep all components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

**Buffer/Protocol Choice:** Terminator 5′-Phosphate-Dependent Exonuclease is a processive 5′→3′ exonuclease that digests RNA that has a 5′ monophosphate. It does not digest RNA that has a 5′-triphosphate, 5′-cap, or 5′-hydroxyl group. However, on rare occasions, we have observed a secondary, non-5′-monophosphate-specific activity on 5′-triphosphate and 5′-hydroxyl RNAs. Because of this activity, two different 10X Reaction Buffers are provided with this kit. The Terminator 10X Reaction Buffer A and its affiliated protocol constitute the standard buffer and protocol. They have been optimized for maximum 5′-monophosphate RNA digestion (e.g., rRNA digestion). The Terminator 10X Reaction Buffer B and its affiliated protocol are provided as an alternate method that should only be used in the rare event that a specific RNA of interest is being degraded by a secondary non-5′-monophosphate-specific activity. Note, however, that less rRNA will be degraded with this buffer and protocol.

***Note:*** *Using the two different buffers in a 50:50 ratio will* ***not*** *produce a reaction condition with the benefits of each individual buffer and protocol.*

## 5. Standard Procedure

***Important:*** *Two different reaction buffers and protocols are included with Terminator Exonuclease. Be sure to read Buffer/Protocol Choice on page 3 to determine which protocol is appropriate for your intended use.*

This protocol uses the Terminator 10X Reaction Buffer A and has been optimized for maximum 5′-monophosphate rRNA digestion (e.g., rRNA digestion).

The RNA sample to be treated should be dissolved in RNase-free water. **Do not** dissolve the RNA sample in TE Buffer.

1. Gently mix and briefly centrifuge the Terminator 10X Reaction Buffer A prior to use.
2. In a sterile (RNase-free) 0.2-ml or 0.5-ml tube, combine the following reaction components on ice:

| | | |
|---|---|---|
| x | µl | RNase-Free Water |
| 2 | µl | Terminator 10X Reaction Buffer A |
| 0.5 | µl | RiboGuard RNase Inhibitor |
| x | µl | Total RNA Sample (200 ng - 10 µg) |
| 1 | µl | Terminator Exonuclease (1 Unit) |
| 20 | µl | Total reaction volume |

3. Incubate the reaction at 30°C for 60 minutes in a thermocycler (with heated lid) or water bath.
4. Terminate the reaction by one of the two methods described in Step 4, page 4.

## 6. Alternative Procedure

***Important:*** *Two different reaction buffers and protocols are included with Terminator Exonuclease. Be sure to read Buffer/Protocol Choice to determine which protocol is appropriate for your intended use.*

This protocol uses the Terminator 10X Reaction Buffer B and should only be used in the rare event that a specific RNA of interest is being degraded by a secondary non-5′-monophosphate-specific activity of the enzyme.

***Note:*** *Less rRNA will be degraded with this buffer and protocol.*

The RNA sample to be treated should be dissolved in RNase-free water. Do not dissolve the RNA sample in TE Buffer.

1. Gently mix and briefly centrifuge the Terminator 10X Reaction Buffer B prior to use.
2. In a sterile (RNase-free) 0.2-ml or 0.5-ml tube, combine the following reaction components on ice:

| | | |
|---|---|---|
| x | µl | RNase-Free Water |
| 2 | µl | Terminator 10X Reaction Buffer B |
| 0.5 | µl | RiboGuard RNase Inhibitor |
| x | µl | Total RNA Sample (1-2.5 µg) |
| 1 | µl | Terminator Exonuclease (1 Unit) |
| 20 | µl | Total reaction volume |

3. Incubate the reaction at 42°C for 30 minutes in a thermocycler (with heated lid) or water bath.
4. Terminate the reaction by one of the two methods described below:

4a. **Terminate the reaction by adding 1 µl of 100 mM EDTA (pH 8.0).** Place the reaction on ice. Note that the enriched mRNA sample will now contain 5 mM EDTA (as well as tRNA, nucleotides and other small RNAs). It may be used directly for applications in which the EDTA will not be inhibitory. However, the high concentration of EDTA may interfere with some subsequent uses of the mRNA, such as RT-PCR. Therefore, it may be necessary to remove the excess EDTA by LiCl precipitation (see below), ethanol precipitation, or use of an RNA purification column.

4b. **Terminate the reaction by phenol extraction and ethanol precipitation.**

1. Add RNase-Free Water to the reaction to a total volume of 200 µl.
2. Extract once with buffer-saturated phenol.
3. Transfer the aqueous phase to a new RNase-free tube.
4. Add 0.1 volume of 3 M sodium acetate and 2.5 volumes of ethanol to the reaction and mix thoroughly.
5. Incubate on ice or at –20°C for 30 minutes.
6. Pellet the RNA by centrifugation in a microcentrifuge for 30 minutes at full speed at 4°C.
7. Carefully remove and discard the supernatant. Do not disturb the RNA pellet which contains the mRNA.
8. Wash the RNA pellet with 70% ethanol to remove residual salt.
9. Resuspend the RNA pellet in RNase-Free Water or TE Buffer.

5. **(Optional) Purify the enriched mRNA.** If desired, the enriched mRNA can be purified from excess EDTA, tRNA, 5S rRNA, and other small RNA species, by LiCl precipitation or by using a commercial RNA purification column.

   **Lithium chloride precipitation should be performed only if the original reaction contains >2 µg of total RNA.** If the amount of total RNA in the reaction was less than 2 µg, purify the mRNA by phenol extraction and ethanol precipitation as described above or using a commercial RNA purification column.

   Lithium chloride selectively precipitates large RNA such as mRNA, while small RNA such as tRNA, and nucleotides and salts (e.g., EDTA) remain in solution.

   1. Add 1 volume of 5 M LiCl solution to the sample and mix well.
   2. Incubate on ice or at –20°C for 30 minutes.
   3. Pellet the RNA by centrifugation in a microcentrifuge for 30 minutes at full speed at 4°C.
   4. Carefully remove and discard the supernatant which contains the tRNA, other small RNAs and nucleotides. Do not disturb the RNA pellet which contains the mRNA.
   5. Wash the RNA pellet with 70% ethanol to remove residual salt.
   6. Resuspend the RNA pellet in RNase-free water or TE buffer.
   6. (Optional) Analyze the effectiveness of the Terminator reaction by denaturing agarose gel electrophoresis or using an Agilent 2100 Bioanalyzer. When using either method, it is important to run an untreated RNA sample (~250 ng of total RNA) alongside the treated sample. The absence of 18S and 28S rRNA in the post-treatment sample indicates a successful reaction.



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