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mRNA-ONLY™ Prokaryotic mRNA Isolation Kit

Cat. Nos. MOP51010 and MOP51024

** Patent pending*

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1. Introduction

The mRNA-ONLY™ Prokaryotic mRNA Isolation Kit* provides a simple and effective method for isolating prokaryotic mRNA that is substantially free of ribosomal RNA (rRNA) in 1 hour. The mRNA-ONLY Kit includes Epicentre's Terminator™ 5′-Phosphate-Dependent Exonuclease, a processive 5′→3′ exonuclease that digests RNA having a 5′ monophosphate. The enzyme does not digest RNA having a 5′ triphosphate, a 5′ cap (present on most eukaryotic mRNAs), or a 5′-hydroxyl group. Bacterial rRNAs which are transcribed as a single transcript and then processed to yield rRNAs with 5′ monophosphates are substrates for the enzyme. Thus, the mRNA-ONLY Prokaryotic mRNA Isolation Kit can be used to isolate prokaryotic mRNA substantially free of 16S and 23S rRNA.

Terminator Exonuclease will also digest single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) 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.

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 of Terminator 5′-Phosphate-Dependent Exonuclease digests 1 µg of rRNA substrate into acid-soluble nucleotides in 60 minutes at 30°C under standard assay conditions.

Contaminating Activity Assays: All components of the mRNA-ONLY Prokaryotic mRNA Isolation Kit are free of detectable contaminating endonuclease and non-5′-monophosphate-dependent nuclease activities.

Quality Control: The mRNA-ONLY Prokaryotic mRNA Isolation Kit is function-tested by treating 5 µg of *E. coli* total RNA with Terminator Exonuclease under standard assay conditions (30°C for 1 hour). Removal of 16S and 23S rRNA is confirmed by denaturing agarose gel electrophoresis.

3. Kit Contents

Desc.	Concentration	Quantity
The mRNA-ONLY™ Prokaryotic mRNA Isolation Kit is available in a 10- and 24-purification size kits. The 10-purification size kit contains:		
Terminator™ 5′-Phosphate-Dependent Exonuclease	(1 U/µl)	10 µl
mRNA-ONLY™ Prokaryotic 10X Reaction Buffer A		25 µl
mRNA-ONLY™ Prokaryotic 10X Reaction Buffer B		25 µl
RiboGuard™ RNase Inhibitor		10 µl
mRNA-ONLY™ Stop Solution		10 µl
5 M LiCl Solution		250 µl
RNase-Free Water		250 µl

4. Related Products

The following products are also available:

- mRNA-ONLY™ Prokaryotic mRNA Isolation Kit with Poly(A)-Tailing
- mRNA-ONLY™ Eukaryotic mRNA Isolation Kit
- MasterPure™ RNA Purification Kit
- MasterPure™ Complete DNA and RNA Purification Kit
- Terminator™ 5′-Phosphate-Dependent Exonuclease
- MonsterScript™ Reverse Transcriptase
- MonsterScript™ 1st-Strand cDNA Synthesis Kit
- MMLV Reverse Transcriptase
- Tobacco Acid Pyrophosphatase
- RNA 5′ Polyphosphatase
- RiboGuard™ RNase Inhibitor

5. General Considerations

RNA Purification Methods and RNA Purity. Total cellular RNA, isolated by a number of methods, can be treated successfully using the mRNA-ONLY Kit. However, it is very important that the purified RNA be free of salts, metal ions, ethanol, and phenol which can inhibit the Terminator Exonuclease. Commonly used RNA extraction and purification methods that are compatible with the mRNA-ONLY Kit process include but are not limited to:

Salt-Fractionation: RNA purification that employs gentle salt-fractionation, such as Epicentre's MasterPure™ RNA Purification Kit, routinely produce the highest yield of intact RNA without the use of phenol, guanidinium salts, or columns. When using the MasterPure kit, be sure to wash the RNA pellet at least two times with cold 70-75% ethanol to remove all traces of salts. Air dry the RNA pellet (do not use a vacuum centrifuge) to remove residual ethanol. Then, resuspend the RNA in RNase-free water. DO NOT dissolve the RNA sample in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA). Total RNA isolated by salt-fractionation methods retains all species of cellular RNA including mRNA, rRNA, tRNA, and other small RNAs. The tRNA and other small RNAs can be removed from the Terminator Exonuclease treated sample by lithium chloride precipitation (see Step 5 in the Procedure). The MasterPure kit can be used for purification of total RNA from cells preserved using RNAprotect™ Bacteria Reagent (Qiagen) but is not recommended for purification of RNA from tissue samples preserved with RNA*later*® or RNA*later*-ICE.

TRIzol®/TRI Reagent®, a homogeneous solution of the powerful denaturants guanidinium isothiocyanate and phenol, is very effective at extracting the RNA from the cells. However all traces of guanidinium salts and phenol must be removed from the RNA sample prior to the treatment. If you precipitate the RNA from TRIzol-extracted cells, be sure to wash the RNA pellet at least two times with cold 70%-75% ethanol to remove all traces of phenol and guanidinium salts. Air dry the RNA pellet (do not use a vacuum centrifuge) to remove residual ethanol. Then, resuspend the RNA in RNase-free water. DO NOT dissolve the RNA sample in TE Buffer. If you purify the RNA from TRIzol-extracted cells by column purification methods, please read the section "Spin Columns" immediately following.

Spin Columns (e.g., the RNeasy® MinElute® Cleanup Kit and RNeasy Mini Kit from Qiagen) are effective in purifying RNA samples and remove contaminants that may inhibit the RNA treatment. Spin columns can be used with most RNA extraction procedures (e.g. TRIzol reagent). If using spin columns, follow the manufacturer's instructions closely, especially if an ethanol wash of the RNA is performed prior to the RNA elution step. Then, elute the RNA from the column membrane using RNase-free water. DO NOT dissolve the RNA sample in TE Buffer. Many spin column purification methods remove the 5S rRNA, tRNAs, and other small RNAs. We recommend using spin columns to isolate RNA from tissue samples treated with RNA preservatives such as RNA*later* or RNA*later*-ICE.

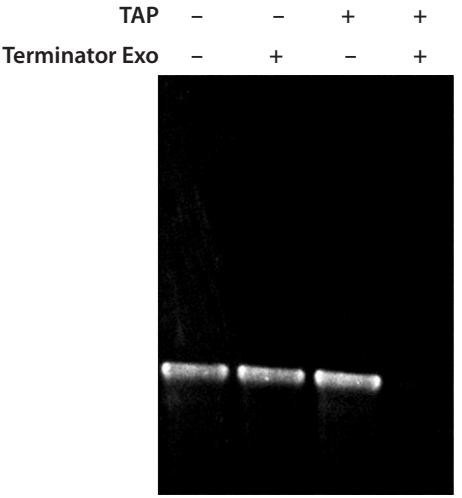

Figure 1. Terminator™ Exonuclease Treatment of 5′-Triphosphate-Containing RNA.

RNA transcripts containing a 5′ triphosphate (representing prokaryotic primary transcripts) were incubated with Terminator Exonuclease before and after treatment with Tobacco Acid Pyrophosphatase (TAP), an enzyme that yields RNA with a 5′ monophosphate from 5′-triphosphorylated RNAs.

Lane 1, untreated RNA.

Lane 2, RNA treated only with Terminator Exonuclease.

Lane 3, RNA treated only with TAP.

Lane 4, RNA treated with both Terminator Exonuclease and TAP.

As shown in Lane 2, 5′-triphosphorylated RNA is recovered intact after Terminator Exonuclease treatment.

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 mRNA-ONLY Prokaryotic 10X Reaction Buffer A and its affiliated protocol constitute the standard buffer and protocol. They have been optimized for maximum rRNA digestion. The mRNA-ONLY Prokaryotic 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. 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.

RNA Integrity. Successful use of the mRNA produced by an mRNA-ONLY Prokaryotic mRNA Isolation Kit reaction can be strongly influenced by the quality of the total RNA sample. A partially degraded RNA sample will contain rRNA and mRNA fragments with 5′-OH ends which are not substrates for the Terminator Exonuclease. These RNA fragments may have an adverse affect on experiments utilizing the enriched mRNA produced using Terminator Exonuclease. Therefore, it is important to prepare and confirm the integrity of the RNA sample prior to beginning the mRNA-ONLY reaction.

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 endoribonuclease that can contaminate any lab environment and is present on human skin. Partial degradation of a total RNA sample by RNase A or an RNase A-like enzyme produces rRNA and mRNA fragments with 5′-OH ends. These fragments will not be removed by the Terminator Exonuclease and may ultimately have an adverse affect on experiments utilizing the enriched mRNA produced using Terminator Exonuclease. Therefore, 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 kit components. Do not pick up any kit component with an ungloved hand.
4) Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

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.*

6. Standard Procedure

Important! *Two different reaction buffers and protocols are included with the mRNA-ONLY kit. Be sure to read General Considerations:Buffer/Protocol Choice to determine which protocol is appropriate for your intended use.*

This protocol uses the mRNA-ONLY Prokaryotic 10X Reaction Buffer A and has been optimized for maximum 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 mRNA-ONLY Prokaryotic 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	mRNA-ONLY Prokaryotic 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 on page 6.

7. Alternative Procedure

Important: *Two different reaction buffers and protocols are included with the mRNA-ONLY kit. Be sure to read General Considerations:Buffer/Protocol Choice to determine which protocol is appropriate for your intended use.*

This protocol uses the mRNA-ONLY Prokaryotic 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 mRNA-ONLY Prokaryotic 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	mRNA-ONLY Prokaryotic 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.

(Optional) Analyze the effectiveness of the mRNA-ONLY reaction. The effectiveness of the mRNA-ONLY reaction can be assessed 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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