CELLSCRIPT™
RNA for Translation in Cells

RNA 5' Polyphosphatase

Cat. No. **R5P2601400**

INTRODUCTION

RNA 5' Polyphosphatase is a patented enzyme* that catalyzes the removal of γ and β phosphate groups from 5'-triphosphorylated and 5'-diphosphorylated RNA resulting in 5'-monophosphorylated RNA. Capped RNA is not a substrate for the enzyme. RNA 5' Polyphosphatase can be used for the following applications:

- Removal of uncapped RNA from an mRNA preparation, in conjunction with XRN1 treatment.[1-4]
- 5' end RNA substrate preparation for RNA Ligase-based circularization reactions.[5,6]
- 5' end RNA substrate preparation for T4 RNA Ligase-based reactions (end tagging).[7]

RNA 5' Polyphosphatase is active on nucleoside triphosphates. Removal of unincorporated nucleotides from completed transcription reactions is recommended for full enzymatic activity on the intended RNA substrate.

RNA 5' Polyphosphatase activity is inhibited by inorganic phosphate, a natural reaction product. Removal of inorganic phosphate from completed transcription reactions is recommended for full enzymatic activity on the intended RNA substrate.

MATERIALS

Materials Supplied

Important Store at –20°C in a freezer without a defrost cycle. Do not store at –70°C.

RNA 5' Polyphosphatase	
Components	Volume
RNA 5' Polyphosphatase, 20 U/µl in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 0.1 mM EDTA and 0.1% Triton® X-100.	20 µl
10X RNA 5' Polyphosphatase Reaction Buffer 0.5 M HEPES-KOH (pH 7.5), 1 M NaCl, 10 mM EDTA, 1% β-mercaptoethanol, and 0.1% Triton X-100.	100 µl

SPECIFICATIONS

Unit Definition

One unit of RNA 5' Polyphosphatase results in the acid-solubilization of 1 nanomole of inorganic phosphate from ATP in 1 hour at 37°C.

Contaminating Activity Assays

RNA 5' Polyphosphatase is free of detectable DNA exo- and endonuclease and RNase activities.

* Covered under patents assigned to CELLSCRIPT Inc.

For more information, consult the appropriate safety data sheet (SDS) at www.cellscript.com.

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BEFORE YOU START: IMPORTANT TIPS FOR OPTIMAL EFFICIENCY

- **RNA Source**:

  RNA produced in an *in vitro* transcription reaction should be purified to remove reaction products such as unincorporated nucleotides and inorganic phosphate and resuspended in RNase-Free Water or RNase-free TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) prior to RNA 5' Polyphosphatase treatment.

- **RNA Secondary Structure**:

  Some RNA transcripts can form stable secondary structures (homodimers and hairpins) involving the 5'-most nucleotides of the transcript severely limiting access of the 5'-most nucleotide to RNA 5' Polyphosphatase. In order to increase the treatment efficiency of such RNAs, use longer or hotter heat denaturation conditions than those noted in the protocol. Times and temperatures required will vary.

PROCEDURE

Two protocols are provided for use of RNA 5' Polyphosphatase. Most RNAs can be treated with the **Standard Protocol** (see Figure 1, page 5). For RNAs that contain significant/stable secondary structure involving the 5' end of the RNA, the **Alternate Protocol** may be required (see Figure 2, page 5).

A1. Standard Protocol: Treatment of Uncapped Primary Transcript (5'-triphosphate) RNA (see Figure 1, page 5).

1. This reaction treats up to **50 µg** of RNA.
   The reaction can be scaled for individual needs.

   Combine the following reagents:

Standard RNA 5' Polyphosphatase Reaction	
Component	Amount
RNase-Free Water	x µl
10X RNA 5' Polyphosphatase Reaction Buffer	2 µl
ScriptGuard™ RNase Inhibitor, 20 Units (sold separately)	0.5 µl
RNA Substrate, ≤ 50 µg	y µl
RNA 5' Polyphosphatase, 20 Units	1 µl
Total Volume	20 µl

Addition of ScriptGuard RNase Inhibitor is optional but highly recommended.

2. Incubate at 37°C for 30 minutes.

3. Stop the reaction using any one of the following methods:
   a) Immediate storage at –20°C or –70°C.
   b) Immediate purification of the sample
   (e.g., organic extraction/nucleic acid precipitation or column purification).

A2. Alternate Protocol: Treatment of Uncapped Primary Transcript (5'-triphosphate) RNA containing significant/stable 5'-end secondary structure (see Figure 2, page 5)**.**

1. This protocol utilizes a pre heat denaturation step to melt out 5'-end secondary structure prior to RNA 5' Polyphosphatase treatment. Higher ratios of enzyme to substrate are required for this protocol.

   This reaction treats up to **5 µg** of RNA.
   The reaction can be scaled for individual needs.

   Combine the following reagents:

Alternate RNA 5' Polyphosphatase Reaction (step 1)	
Component	Amount
RNase-Free Water	x µl
RNA Substrate, ≤ 5 µg	y µl
Total Volume	16.5 µl

Important Only heat-denature the RNA and water components. Do not include any other reagent in this step.

2. Incubate at 65°C for 5-10 minutes, then transfer to ice.

3. While the heat-denatured RNA is cooling on ice, prepare a "Cocktail" of the following reaction components together in a separate tube.

Alternate RNA 5' Polyphosphatase Reaction (step 3)	
Component	Amount
10X RNA 5' Polyphosphatase Reaction Buffer	2 µl
ScriptGuard™ RNase Inhibitor, 20 Units (sold separately)	0.5 µl
RNA 5' Polyphosphatase, 20 Units	1 µl
Total Volume	3.5 µl

Addition of ScriptGuard RNase Inhibitor is optional but highly recommended.

4. Combine the following reagents:

Alternate RNA 5' Polyphosphatase Reaction (step 4)	
Component	Amount
Heat-denatured RNA (from step 1)	16.5 µl
Cocktailed reaction components (from step 2)	3.5 µl
Total Volume	20 µl

5. Incubate at 37°C for 30 minutes.

3. Stop the reaction using any one of the following methods:
   a) Immediate storage at –20°C or –70°C.
   b) Immediate purification of the sample
   (e.g., organic extraction/nucleic acid precipitation or column purification).

B1. Standard Protocol: Example Data

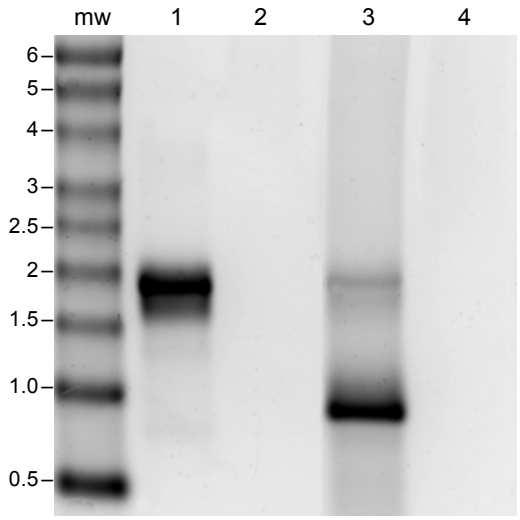


Figure 1 Standard Protocol

Two different 5'-triphosphate primary transcript RNAs were treated with RNA 5' Polyphosphatase using the Standard Protocol (no pre heat denaturation step used). After the 30 minute incubation, additional $MgCl_2$ (for buffer compatibility) and XRN1 (5'-monophosphate-dependent exonuclease) was added to the reaction to digest the RNA 5' Polyphosphatase reaction product.

mw) RNA molecular weight markers (kb)
1) 1.85-kb RNA, treated with XRN1-only
2) 1.85-kb RNA, treated with both RNA 5' Polyphosphatase and XRN1
3) 0.92-kb RNA, treated with XRN1-only
4) 0.92-kb RNA, treated with both RNA 5' Polyphosphatase and XRN1

B2. Alternate Protocol: Example Data

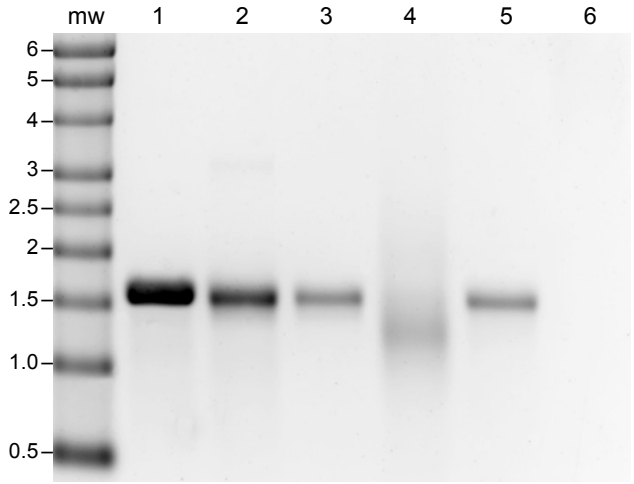


Figure 2 Alternate Protocol

A 1.57-kb 5'-triphosphate primary transcript RNA containing stable secondary structure on the 5' end was treated with RNA 5' Polyphosphatase using the Alternate Protocol (pre heat denaturation step used). After the 30 minute incubation, additional $MgCl_2$ (for buffer compatibility) and XRN1 (5'-monophosphate-dependent exonuclease) was added to the reaction to digest the RNA 5' Polyphosphatase reaction product.

mw) ssRNA molecular weight markers (kb)
1) Untreated 1.57-kb IVT RNA
2) Treated with XRN1-only (+pre heat denaturation)
3) Treated with RNA 5' Polyphosphatase (–pre heat denaturation) and XRN1 (–pre heat denaturation)
4) Treated with RNA 5' Polyphosphatase (–pre heat denaturation) and XRN1 (+pre heat denaturation)
5) Treated with RNA 5' Polyphosphatase (+pre heat denaturation) and XRN1 (–pre heat denaturation)
6) Treated with RNA 5' Polyphosphatase (+pre heat denaturation) and XRN1 (+pre heat denaturation)

RELATED PRODUCTS

– T4 RNA Ligase 2
– ScriptGuard™ RNase Inhibitor

REFERENCES

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6. Hang, R. et al., (2015) Bio-Protocol Journal, 5 (14). doi: 10.21769/BioProtoc.1533.
7. Vvedenskaya, I. et al., (2018) Methods Enzymol., 612, 269. doi: 10.1016/bs.mie.2018.09.007.

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