1. Introduction

HyperScribe™ Poly (A) Tailing Kit is a set of reagents designed to add a ≥150 base poly (A) tail to RNA transcripts generated with the HyperScribe™ T7 High Yield RNA Synthesis Kit. The kit is completed by using *E. coli* Poly (A) Polymerase (*E*-PAP) and ATP. Then the resulting capped and tailed RNA can be used in transfection or micro-injection experiments where mRNA stability and translation efficiency may be increased relative to unmodified mRNA.

2. Materials

2.1. Components and storage

Reagents for 25 reactions

Amount	Component	Storage
100 µL	*E*-PAP (2 units/µL)	–20°C
600 µL	5X *E*-PAP Buffer	–20°C
100 µL	ATP Solution (100 mM)	–20°C
250 µL	25 mM MnCl2	–20°C
2x1mL	Nuclease-free Water	any temp*

* Store Nuclease-free Water at –20°C, 4°C, or room temperature.

For storage at -20℃, use a frost-free refrigerator. When using the kit, keep the reagents on ice; 10 mM ATP and *E*-PAP are particularly labile.

2.2. Materials Not Supplied

For the positive control reaction: HyperScribe™ T7 High Yield RNA Synthesis Kit (K1047)

3. Poly(A) Tailing Procedure

The reactions described below add a≥150 base poly (A) tail to transcripts generated with the HyperScribe™ T7 High Yield RNA Synthesis Kit. *To produce a tail shorter than ~150 bases*, dilute *E*-PAP in 1X *E*-PAP buffer and use less *E*-PAP in the reaction. (For 1X *E*-PAP buffer, dilute 5X *E*-PAP buffer with nuclease - free water.)

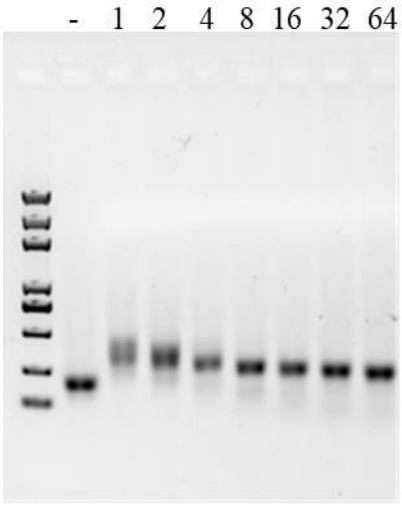

Figure 1. Titration of *E*-PAP into a Tailing Reaction.

The Control DNA Template was transcribed in a HyperScribe™ T7 High Yield RNA Synthesis reaction. The resulting 130 nt transcript was tailed with decreasing amounts of *E*-PAP. - indicates untailed transcripts; 1, 2, 4, 8, 16, 32 and 64 represent the dilution factor.

3.1. Tailing reaction

(a) Start with a completed, DNase-treated HyperScribe™ T7 High Yield RNA Synthesis reaction (20 µL in a 1.5 mL tube) at room temperature. Do ***not*** add EDTA to the reaction as is sometimes recommended to inactivate DNase.

(b) At room temperature, add the tailing reagents in the order shown to a 20 µL HyperScribe™ T7 High Yield RNA Synthesis reaction:

Amount	Component
20 µL	HyperScribe™ T7 High Yield RNA Synthesis reaction
42 µL	Nuclease-free Water
20 µL	5X *E*-PAP Buffer
10 µL	25 mM MnCl2
4 µL	100 mM ATP

(c) Remove 0.5 µL of the reaction mixture above; this minus-enzyme control will be run on a gel next to the tailed RNA at the end of the experiment.

(d) Add 4 µL of *E*-PAP, and mix gently. The final reaction volume is 100 µL.

(e) Incubate at 37 ℃ for 1 h.

(f) Keep reaction on ice or store at –20 ℃.

3.2. Denaturing agarose gel electrophoresis

(a) During the 1 hour incubation, pour a denaturing agarose-formaldehyde gel of the appropriate

percentage for the size of your original (untailed) transcript. Use a 0.75 mm (or thinner) comb for optimal resolution.

Transcription size	
Larger than 500bp	1% argarose
Smaller than 500bp	1.5% argarose

(b) Prepare an aliquot of each tailing reaction, and the corresponding minus-enzyme control. Also plan to run an RNA size marker.

In a 0.5 mL RNase-free microcentrifuge tube mix 4 μL gel loading dye containing 50 μg/mL gel red with 0.5 μL RNA sample.

NOTE: The gel loading dye must contain ~20 mM EDTA to chelate divalent cations from the tailing reaction reagent. Without EDTA, divalent cations can cause RNA degradation when RNA is denatured by heating.

(c) Heat samples at 75 ℃ for 10 min.

(d) Load the samples, and run the gel in 1X MOPS buffer at~5 volts/cm until the bromophenol blue dye is near the bottom of the gel.

(e) Examine the gel on a UV light box. The tailed RNA should be ≥150 bases longer than the corresponding RNA that was not tailed (minus-enzyme control).

3.3. (optional) Remove unincorporated nucleotides

Remove free nucleotides from the RNA with the MEGAclear™ Transcription Clean-Up Kit (AM1908, Thermo Fisher) by gel filtration [for example with a NucAway™ Spin Columns(AM10070, Thermo Fisher)]or by precipitation with LiCl or ammonium acetate.

Lithium chloride precipitation

Lithium Chloride (LiCl) precipitation is a convenient and effective way to remove unincorporated nucleotides and most proteins. Lithium chloride precipitation, however, does not precipitate transfer RNA and may not efficiently precipitate RNAs smaller than 300 nucleotides. Also, the concentration of RNA should be at least 0.1 μg/μL to assure efficient precipitation.

(a) Stop the reaction and precipitate the RNA by adding 30 μL Nuclease-free Water and 30 μL LiCl Precipitation Solution.

(b) Mix thoroughly. Chill for ≥30 min at –20℃.

(c) Centrifuge at 4 ℃ for 15 min at maximum speed to pellet the RNA.

(d) Carefully remove the supernatant. Wash the pellet once with ~1 mL 70% ethanol, and re-centrifuge to maximize removal of unincorporated nucleotides.

(e) Carefully remove the 70% ethanol, and resuspend the RNA in a solution or buffer appropriate for your application. Determine the RNA concentration and store frozen at –20 ℃ or –70 ℃.

3.4. Quantitation of tailed RNA

If the unincorporated nucleotides have been removed, the amount of RNA is determined by reading its absorbance at 260 nm. If unincorporated nucleotides have not been removed, the RNA concentration is estimated by comparing the ethidium bromide signal on the gel band to known standards.

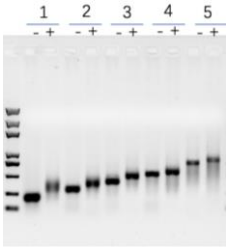

Figure 2. Tailing Transcripts of Different Sizes

Transcripts of 130bp, 290bp, 520bp, 900bp and 1.8 kb were generated from HyperScribe™ T7 High Yield RNA Synthesis reactions and each RNA was tailed using the Poly (A) Tailing Kit according to the protocol. -: indicates untailed transcripts; +: indicates tailed transcripts.

4. Notes

4.1. Positive Control Reaction

(a) Transcribe 2 µL EGFP control template using the the HyperScribeTM T7 High Yield RNA Synthesis Kit. Do not add radiolabeled UTP tracer to the transcription reaction.

(b) Treat the transcript with DNase I as described in the HyperScribeTM T7 High Yield RNA Synthesis Kit Protocol.

(c) Add a poly(A) tail to the transcript according to the instructions in section **3** on page 1 of this Protocol, but add 1 µL of [α-^{32}P] ATP (any specific activity can be used). Tracking the radiolabel makes it relatively easy to determine how much ATP was incorporated into poly (A) at the end of the reaction. Remember to remove an aliquot of reaction mixture before the addition of *E*-PAP as a minus-enzyme control (section **3.1. (c)** on page 2).

(d) Determine portion of the labeled incorporated into RNA by TCA precipitation. ***Successful reactions incorporate 50% or more radiolabel.***

(e) Run 0.5 µL of the positive control reaction product and the corresponding minus-enzyme control on a 2.5% formaldehyde-agarose gel with RNA markers as described in step 3.2.on page 2.
The tailed reaction should produce a band that is ≥150 bases longer than the minus-enzyme control.

4.2. No RNA visible on the gel

(a) Problem with the transcription reaction

Refer to the HyperScribeTM T7 High Yield RNA Synthesis Kit Protocol for notes.

(b) RNase contamination

Treat all equipment, gel boxes etc. with RNase*Zap*® Solution and use RNase-free tubes and reagents.

4.3. No visible size shift seen after tailing

(a) Check the kit components by doing the positive control reaction

Verify that the positive control reaction incorporates at least 50% of the radiolabeled ATP.

(b) The positive control works, but the experimental transcript does not

Inadequate gel resolution:

If the experimental transcript is several kb, then the resolution of an agarose gel may not be adequate to resolve a size change. Check the reaction by trace labeling instead (see the next section).

Trace label the tailing reaction

If the positive control reaction product produces a larger band than the minus-enzyme control, but the experimental transcript does not, then add a trace label to the tailing reaction, and measure the incorporation of label by TCA precipitation.

If less than 50% of the label is incorporated, but radiolabel incorporation is greater than background, increasing either the amount of enzyme or the concentration of ATP may help to generate a poly (A) tail of the desired length.