Poly(A) Tailing Kit

For Polyadenylation of RNA
Part Number AM1350

Applied Biosystems

A. Product Description

The Poly(A) Tailing Kit is a set of reagents designed to add a ≥150 base poly(A) tail to RNA transcripts generated with the Ambion® mMESSAGE mMACHINE® Kit. This is accomplished using *E. coli* Poly(A) Polymerase (*E*-PAP) and ATP. The resulting capped and tailed RNA can then be used in transfection or micro-injection experiments where enhanced translation over untailed mRNAs may be seen due to increased mRNA stability and translation efficiency (see *F. References* on page 7).

B. Kit 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
250 µL	ATP Solution (10 mM)	–20°C
250 µL	25 mM $MnCl_2$	–20°C
10 µL	Control DNA Template (0.5 µg/µL)	–20°C
2 x 1 mL	Nuclease-free Water	any temp*

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

For storage at –20°C, use a non-frost free freezer. Keep reagents on ice while using the kit; the 10 mM ATP and the *E*-PAP are especially labile.

C. Materials Not Provided with the Kit

Reagents and equipment to run denaturing agarose gels:

- Electrophoresis reagents: Applied Biosystems offers gel loading dyes, agaroses, acrylamide solutions, gel buffer mixes, and nuclease-free water for electrophoresis. Please see the catalog (print or web) for a complete list as this product line is always growing.
- RNA Century™-Plus Markers (P/N AM7145) or RNA Millennium™ Markers (P/N AM7150)

For the positive control reaction:

- mMESSAGE mMACHINE® Kit (P/N AM1340, AM1344, AM1348)
- [α-^{32}P]ATP (any specific activity can be used)

D. Poly(A) Tailing Procedure

The reaction described below adds a ≥150 base poly(A) tail to transcripts generated with the Ambion mMESSAGE mMACHINE Kit. ***To generate tails 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.)

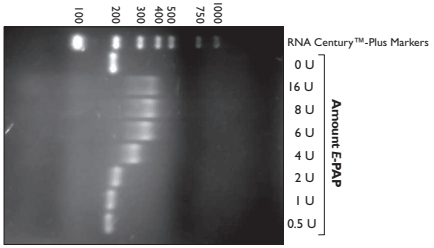

Figure 1. Titration of *E*-PAP into a Tailing Reaction.
The Control DNA Template was transcribed in a mMESSAGE mMACHINE Kit reaction. The resulting 188 nt transcript was tailed with decreasing amounts of *E*-PAP.

For the following hazards, see the complete safety alert descriptions in section H starting on page 8.

CHEMICAL HAZARD. 25 mM $MnCl_2$, 5X E-PAP Buffer, E-PAP.

1. Tailing reaction

a. Start with a completed, DNase-treated mMESSAGE mMACHINE 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 mMESSAGE mMACHINE reaction:

Amount	Component
20 µL	mMESSAGE mMACHINE reaction
36 µL	Nuclease-free Water
20 µL	5X *E*-PAP Buffer
10 µL	25 mM $MnCl_2$
10 µL	10 mM ATP

c. Remove 0.5 µL of the reaction mixture before adding the *E*-PAP enzyme; 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°C for 1 hr.

f. Place reaction on ice or store at –20°C.

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 (e.g. using NorthernMax® reagents). Use a 0.75 mm (or thinner) comb for optimal resolution.

Transcript size	
smaller than 500 bases	2.5% agarose
larger than 500 bases	1% agarose

b. Prepare an aliquot of each tailing reaction, and the corresponding minus-enzyme control. Also plan to run an RNA size marker (for example, Ambion RNA Century-Plus Markers, P/N AM7145, or Millennium Markers, P/N AM7150).

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

IMPORTANT

The gel loading dye must include ~20 mM EDTA (for example, P/N AM8552) to chelate the divalent cations from the tailing reaction reagents. Without EDTA, divalent cations can cause degradation when the RNA is heat denatured.

c. Heat samples at 75°C 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. (optional) Remove unincorporated nucleotides

Remove free nucleotides from the RNA with the Ambion MEGAclear™ Kit (P/N AM1908), by gel filtration [for example, with an Ambion NucAway™ Spin Column (P/N AM10070)], or by precipitation with LiCl or ammonium acetate as described in the mMESSAGE mMACHINE protocol.

4. Quantitation of tailed RNA

If unincorporated nucleotides have been removed, quantitate the RNA by reading its absorbance at 260 nm. If unincorporated nucleotides have not been removed, estimate the concentration by comparing the ethidium bromide signal of the band on a gel to a known standard.

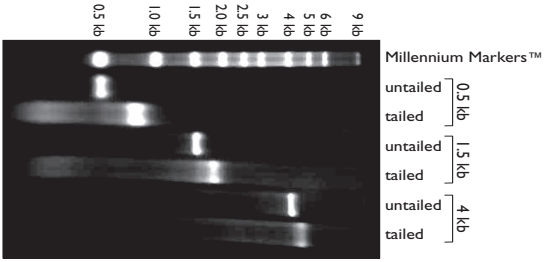

Figure 2. Tailing Transcripts of Different Sizes

Transcripts of 0.5 kb, 1.5 kb and 4 kb were generated from mMESSAGE mMACHINE reactions and each RNA was tailed using the Poly(A) Tailing Kit according to the protocol. The RNA was run on 1% denaturing agarose stained with ethidium bromide.

E. Troubleshooting

1. Positive Control Reaction

a. Transcribe 2 µL pTri-β-actin-125 control template using the Ambion mMESSAGE mMACHINE Kit; any of the available RNA polymerases can be used because the pTri-β-actin-125 template contains all three RNA polymerase promoters in tandem. Do not add radiolabeled UTP tracer to the transcription reaction.

b. Treat the transcript with DNase I as described in the mMESSAGE mMACHINE protocol.

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

d. Determine the fraction of label incorporated into RNA by TCA precipitation as described in the *Additional Procedures* section of the mMESSAGE mMACHINE Kit Protocol.
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 2 on page 3.
The tailed reaction should produce a band that is ≥150 bases longer than the minus-enzyme control.

2. No RNA visible on the gel

Problem with the transcription reaction

Refer to the mMESSAGE mMACHINE Protocol for trouble-shooting.

RNase contamination

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

3. No visible size shift seen after tailing

Check the kit components by doing the positive control reaction

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

The positive control works, but the experimental transcript does not

a. 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).

b. 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. (See section E.1.c on page 6 for trace labeling instructions, and the *Additional Procedures* section of the mMESSAGE mMACHINE Kit Protocol for 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.

F. References

Bernstein P and Ross J (1989) Poly(A), poly(A) binding protein and the regulation of mRNA stability. *Trends Biochem Sci* **14**: 373–377.

Gallie DR (1991) The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes Dev* **5**: 2108–2116.

Harland R and Misher L (1988) Stability of RNA in developing embryos and identification of a destabilizing sequence in TFIIIA messenger RNA. *Development* **102**: 837–852.

Khaleghpour K, et al. (2001) Translational repression by a novel partner of human poly(A) binding protein, Paip2. *Molecular Cell* 7: 205–216.

G. Quality Control

Functional QC

The Control DNA Template is subjected to the positive control reaction procedure described in this booklet. To verify tailing, the sizes of the reaction products before and after the poly(A) tailing reaction are evaluated on a denaturing agarose gel.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

Meets or exceeds specification when a sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

Protease testing

Meets or exceeds specification when a sample is incubated with protease substrate and analyzed by fluorescence.

H. Safety

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety goggles, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.

- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At **www.appliedbiosystems.com**, select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At **www.ambion.com**, go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com), telephone (650-554-2756; USA), or fax (650-554-2252; USA) your request, specifying the catalog or part number(s) and the name of the product(s). The associated MSDSs will be e-mailed unless you request fax or postal delivery. Requests for postal delivery require 1 to 2 weeks for processing.

Note: For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

General alerts for all chemicals

Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Specific chemical alerts

WARNING

5X E-PAP Buffer is harmful if swallowed. Causes eye, skin, and respiratory tract irritation. Avoid breathing vapor.

WARNING

25 mM $MnCl_2$ may impair fertility in men and cause harm to unborn child.

WARNING

E-PAP causes eye, skin, and respiratory tract irritation. May be harmful if swallowed. Avoid breathing vapor.

Manual 1350M Revision C **Revision Date: February 27, 2009**

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