User Manual

Version 4.1

Product name: RNA Poly(A) Tailing Kit

Cat #: RPTK-100, RPTK-200

Description:
The RNA Poly(A) Tailing Kit provides a highly pure and active enzyme and reagents for quickly and easily adding a poly(A) tail to the 3´ end of any RNA. The MCLAB's newly engineered Poly(A) Polymerase uses ATP as a substrate for a template-independent addition of adenosine monophosphate to the 3´-hydroxyl termini of RNA molecules. The Poly(A) Polymerase is encoded by an *E. coli* gene and overexpressed in the host strain.

Applications:
- Addition of a poly(A) tail to an RNA molecule or a mixture of RNA molecules in order to provide a priming site for synthesis of first-strand cDNA using a primer with poly(dT) on its 3´ end.
- Addition of a poly(A) tail to RNA synthesized *in vitro* in order to increase the stability of RNA and enhance its ability to be translated in *vivo* after transfection or micro-injection into eukaryotic cells.
- Synthesis of polyadenylated RNA for nucleic acid amplification or gene expression studies.
- 3´-end labeling RNA or quantifying mRNA.

Recommended Storage Condition: -20°C

List of Components:
The RNA Poly(A) Tailing Kit is supplied in 20 or 100 tailing reactions of 20 µL each.

RNA Poly(A) Tailing Kit 20-rxn size:
Tailing Enzyme Mix 20 µL
5X Reaction Buffer 80 µL
10 mM ATP solution 40 µL

RNA Poly(A) Tailing Kit 100-rxn size:
Tailing Enzyme 100 µL
5X Reaction Buffer 400 µL
10 mM ATP solution 200 µL

Protocol:
The following protocol is designed as a general guideline for generating a poly(A)-tail length of ~100 bases. Customizing reaction to reach a desired tailing length could be achieved by adjusting such as enzyme concentration and/or incubation time.

1. Set up the reaction in a PCR tube on ice as below:

Component	Quantity *	Note
5X Reaction Buffer	4 μL	1x
Nuclease-free water	To 20 μL	
10mM ATP	2 μL	1mM
Tailing Enzyme Mix	1 μL	
RNA Template	x μL	1-10μg

*: For multiple reactions, master mix should be made with 5% extra reagents to reduce pipette error.

2. Gently mix thoroughly and then centrifuge briefly.
3. Incubate the tube at 37°C for 20 minutes
4. Stop the reaction by appropriate methods according to downstream applications, such as –20°C storage, EDTA addition or through a common purification process.

References:

1. Drummond, D.R. et al. (1985) J. Cell. Biol. 100, 1148.
2. Galili, G. et al. (1988) J. Biol. Chem. 263, 5764.
3. Lingner, J. and Keller, W. (1993) Nucleic Acids Res. 21, 2917.