Poly(A) Polymerase, E. coli

Cat. No. E099

Store at -20°C.

Component	Quantity	Cat. No.
Poly(A) Polymerase, E. coli	25 µl	E099-1
10X Poly(A) Polymerase, E. coli Reaction Buffer	1.0 ml	E099-2
ATP (10 mM)	50 µl	E099-3

Description

abm's Poly(A) Polymerase, E. coli (E-PAP) catalyzes the template independent addition of adenosine residues to the 3' termini of RNA resulting in a poly(A) tail. Unlike in Yeast, the E. coli enzyme's activity appears to be sequence independent, requiring no specific recognition sequences. This leads to the rapid poly(A) addition at virtually all unprotected 3' RNA termini regardless of secondary structure.

Protocol

1. Add the following components into a sterile tube and mix gently.

Component	Volume
RNA	1-10 µg
10X Poly(A) Polymerase, E. coli Reaction Buffer	2 µl
ATP (10 mM)	2 µl
Poly(A) Polymerase, E. coli (1 U/µl)	1 µl
RNase-Free ddH_2O	Up to 20 µl

2. Incubate reaction at 37°C for 30 min.
3. Stop reaction by immediately proceeding to a clean-up step. **abm** recommends using RNA Purification Magnetic Beads (Cat. No. G971).

General Notes

- Poly(A) tail length is dependent on several factors including: the molar concentration of RNA 3' hydroxyl ends, incubation time, ATP concentration and amount of enzyme.
- One unit is defined as the amount of enzyme that will incorporate 1 nmol of AMP into RNA in a 20 µl volume in 10 min at 37°C.
- One unit of enzyme incubated with 1-10 µg of RNA in a 20 µl reaction at 37°C for 30 min should generate a poly(A) tail of >100 bases.