

Manual

Product name: Poly(A) Polymerase, Yeast

Cat #: PAPY-100, PAPY-200, PAPY-OEM, B-PP5

Description:

Poly(A) Polymerase catalyzes the template independent addition of AMP from ATP to the 3' end of RNA. Yeast Poly(A) Polymerase works more efficiently than *E. coli* poly(A) polymerase for RNA oligonucleotide labeling and poly(A) tailing. Less incubation time is required for the yeast enzyme. This enzyme labels both long and short substrates. Poly(A) polymerase preferentially labels longer RNA molecules, whereas short RNA molecules are labeled more efficiently by T4 RNA ligase. The reaction requires Mn^{2+} or Mg^{2+} , ATP as substrate, and any RNA containing 3' hydroxyl termini as primer. Longer RNA molecules are somewhat better primers than short oligomers. Substitution of cordycepin 5' triphosphate (3'-dATP) for ATP results in the addition of a single 3' dA residue to the ends of the RNA, a useful technique for labeling RNA at the 3' end.

Poly(A) Tailing of RNA Protocol

Mix the followings:

5X Poly(A) Polymerase Reaction Buffer 5 μ l

RNA 0.2 μ M

ATP 25 mM* 0.5 μ l

Poly(A) Polymerase >5 units

RNase-Free Water to a final volume of 25 μ l

* Radiolabeled, biotinylated or fluorescently-labeled ATP can be added into the reaction.

Incubate at 37°C for 10 to 20 minutes. Terminate the reaction by heating at 65°C for 10 minutes or by adding 5 mM EDTA.