

Poly(A) Polymerase, Yeast

Product number 74225Y/Z

Poly(A) Polymerase (PAP) catalyzes the template-independent addition of adenosine residues from ATP to the 3'-end of RNA. PAP can be used to add poly(A) tails to all classes of RNA, provided they have 3'-hydroxyl termini. PAP can also add poly(U) and poly(G) to poly-adenylated RNA, but with reduced efficiency. Addition of a poly(A) tail to RNA is used for cloning or affinity purification with oligo-dT, enhancing stability, and preparing 3'-end labeled RNA. Substitution of cordycepin-5'-triphosphate for ATP results in addition of a single dA residue to the 3'-end of RNA.

5X Poly(A) Polymerase Reaction Buffer (PN 74226):

100 mM Tris-HCl, pH 7.0, 3.0 mM MnCl₂, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml acetylated BSA, 50% glycerol

Poly(A) Polymerase and 5X Poly(A) Polymerase Reaction Buffer are functionally tested in the following protocol for 3'-end labeling of RNA with cordycepin-5'-triphosphate:

3'-End labeling of RNA

Combine the following:

5X Poly(A) Polymerase Reaction Buffer	2 µl
RNA	0.2 µM
Cordycepin-5'-Triphosphate (or α- ³² P labeled cordycepin-5'- triphosphate, 10 mCi/ml, 5000 Ci/mmol)	0.4 µM
Poly(A) Polymerase	600 units
RNase-Free Water (PN 70783) to make final volume 10 µl	___ µl

Mix the contents and centrifuge briefly. Incubate at 37°C for 10 minutes. Add 40 µl of TE buffer. Extract with phenol:chloroform (1:1) and with chloroform.

Precipitate the labeled RNA with ethanol, wash the pellet with 70% ice cold ethanol and resuspend in TE for further analysis.

Poly(A) tailing of RNA

Mix the following:

5X Poly(A) Polymerase Reaction Buffer	5 µl
RNA	0.2 µM
ATP, 25 mM*	0.5 µl
Poly(A) Polymerase	600 units
RNase-Free Water (PN 70783) to make final volume 25 µl	___ µl

*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.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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23 January 2017

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