Poly(A) Polymerase, Yeast

Cat. No. E017

Store at -20°C.

Product Description

Poly(A) Polymerase catalyses the template independent addition of adenosine residues onto the 3' ends of polyribonucleotides. The use of ATP as a substrate leads to poly(A) tailing whereas substitution of cordycepin-5'-triphosphate (3'- dATP) for ATP results in addition of a single dA residue to the 3'-termini of the RNA. Neither ADP nor dATP can be used as substrates for this enzyme. Poly(A) Polymerase from yeast has been shown to be more effective at oligonucleotide-labeling and poly(A) tailing of long RNA templates than Poly(A) Polymerase from *E. coli*.

Product Component	Quantity	Part No.
Poly(A) Polymerase, Yeast (1 U/µl)	100 µl	E017-1
5X Poly(A) Polymerase, Yeast Reaction Buffer	1 ml	E017-2
25 mM $MnCl_2$	500 µl	E017-3
ATP (10 mM)	150 µl	E017-4

Product Applications

- Labelling of RNA with ATP or cordycepin
- Poly(A) tailing of RNA for cloning or affinity purification
- Increasing translation of RNA transferred into eukaryotic cells

Protocol

3'-End labeling of RNA

1. Add the following components to a sterile tube sitting on ice:

Product Component	Volume
RNA	Variable
Cordycepin-5'-Triphosphate	Variable
Poly(A) Polymerase, Yeast (1 U/µl)	1 µl
5X Poly(A) Polymerase, Yeast Reaction Buffer	2 µl
25 mM $MnCl_2$	1 µl
Nuclease-Free H_2O	up to 10 µl

2. Collect all components by a brief centrifugation. Incubate the reaction at 37°C for 10 minutes.
3. The 3'-End labelled RNA product is ready for immediate downstream applications or for long-term storage at -80°C.

Poly(A) tailing of RNA

1. Add the following components to a sterile tube sitting on ice:

Product Component	Volume
RNA	Variable
ATP (10 mM)*	1.25 µl
Poly(A) Polymerase, Yeast (1 U/µl)	1 µl
5X Poly(A) Polymerase, Yeast Reaction Buffer	5 µl
25 mM $MnCl_2$	2.5 µl
Nuclease-Free H_2O	up to 25 µl

** Radiolabelled, biotinylated or fluorescently-labeled ATP can be substituted in the reaction.*

2. Collect all components by a brief centrifugation. Incubate the reaction at 37°C for 10 to 20 minutes.
3. Terminate the reaction by heating at 65°C for 20 minutes or by adding 5 mM EDTA.
4. The Poly(A)-tailed RNA product is ready for immediate downstream applications or for long-term storage at -80°C.

General Notes

- For heat inactivation, 65°C for 20 minutes.
- Store all components at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for one year from the date of shipping when stored and handled properly.

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