Tribioscience

Poly(A) Polymerase, Yeast

Catalog	Unit
TBP0178	100 µl

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.

Component

Product Component	Quantity
Poly(A) Polymerase, Yeast (1 U/µl)	100 µl
5X Poly(A) Polymerase, Yeast Reaction Buffer	1 ml
25 mM $MnCl_2$	500 µl
ATP (10 mM)	150 µl

Store at -20°C.

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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