E.coli Poly (A) Polymerase

Please read the manual carefully before use.

Cat. No. LA101

Version No. Version 1.0

Storage: at -20℃ for two years

Concentration: 5000 units/ml

Description

This product is purified from *E. coli* after inducible expression of a recombinant vector containing Poly (A) Polymerase gene. It has a molecular weight of 50.2 kDa. The reaction of *E. coli* Poly (A) Polymerase is template-independent. It catalyzes the addition of AMP converted from ATP to the 3'end of the RNA, that is, adding a poly (A) tail to the RNA.

Highlights

Template independent addition of a poly (A) tail to the 3'end of the RNA.

Application

Add a poly(A) tail for reverse transcription into cDNA; labeling RNA with modified ATP or its analogues.

Kit Contents

Component	LA101-01	LA101-02
E.coli Poly (A) Polymerase	100 units	500 units
10×*E.coli* Poly (A) Polymerase Reaction Buffer	80 μl	400 μl
10×DNA Loading Buffer	1 ml	1 ml

Unit definition:

One unit is defined as the amount of enzyme required to incorporate 1 nmol of AMP into RNA in a 50 μl reaction system in 10 minutes at 37°C.

Quality Control:

No DNase, RNase and phosphatase contamination, no DNA or RNA residues in agarose electrophoresis detection.

RNase activity:

In a 20 μl reaction system, 10 units of enzyme are incubated with 50 ng of ssRNA at 37°C. After 2 hours, <10% of ssRNA is degraded.

Exonuclease activity:

In a 50 μl reaction system, 10 units of enzyme are incubated with 1 μg of [3H] labeled DNA ((purified PCR products) at 37°C for 4 hours, and < 1% total radioactivity is released.

Endonuclease activity:

In a 50 μl reaction system, 10 units of enzyme are incubated with 1 μg of pUC19 at 37°C. After 2 hours, <10% of linear DNA is observed.

Phosphatase activity:

In a 100 μl reaction system containing 1 M diethanolamine, 0.5mM MgCl2, 2.5mM disodium p-nitrophenylphosphate, 20U of *E. coli* Poly (A) Polymerase are incubated at 37°C. After 2 hours, i less than 0.001 U of phosphatase is measured.

Storage Buffer:

20 mM Tris-HCl pH 7.4, 300 mM NaCl, 1 mM DTT, 0.5 mM EDTA, 0.15% Triton-X100, 50% Glycerol

10×*E.coli* Poly (A) Polymerase Buffer:

300 mM Tris-HCl pH 7.9, 2.5 mM NaCl, 120 mM $MgCl_2$, 10 mM DTT, 100 mM ATP,

Reaction mix (20 μl Reaction):

Component	Volume
RNA	≤10 μg
10×*E.coli* Poly (A) Polymerase Reaction Buffer	2 μl
E.coli Poly (A) Polymerase	0.5-1 μl
RNase-free Water	Variable
Total volume	20 μl

Reaction condition

Incubate for 10-20 minutes at 37 ℃ and terminate the reaction by adding 10×DNA loading buffer to 1× final concentration or heating at 65 ℃ for 20 minutes.

Notes

• 2.5-5 unites *E. coli* Poly (A) Polymerase can obtain 60-90 nt Poly (A) products. Please adjust the enzyme volume appropriately according to the length of the target product.

FOR RESEARCH USE ONLY