Poly(A) RNA Polymerase

REF: EG25108-S/M

Storage Condition

Store at -20℃ for 2 years.

Components

Component	EG25108S	EG25108M
Poly(A) RNA Polymerase (5 U/μl)	20 μl	100 μl
10× PAP Buffer	1 ml	1 ml
ATP (10 mM)	200 μl	200 μl

Description

Poly(A) Polymerase is a polymerase derived from *Escherichia coli*, also known as E.coli PAP, PAP enzyme, Poly(A) tailing enzyme, or PolyA tailing enzyme. Poly(A) Polymerase catalyzes the rapid and efficient addition of a poly(A) tail to the 3' end of single-stranded RNA (ssRNA) in the presence of ATP. It can use various single-stranded RNA as substrate, while double-stranded RNA and excessively short oligonucleotides (<20 nt) are not recommended as substrates. DNA cannot be used as substrates for this enzyme. Poly(A) tailing reaction catalyzed by *E. coli* Poly(A) Polymerase can only use ATP, but not ADP or dATP. In addition, when CTP and UTP are used, the incorporation amount is less than 5% of that when using ATP. GTP can not be used by this enzyme.

Definition of Activity Unit

One unit is defined as the amount of enzyme that will incorporate 1 nmol of AMP into RNA in a 20 μl volume in 10 minutes at 37℃ .

Applications

1. poly(A) tailing of RNA for cloning or affinity purification.
2. Enhances translation of RNA transferred into eukaryotic cells.
3. 3' labeling of RNA.
4. cDNA synthesis, add poly(A) tails to miRNA, followed by reverse transcription to obtain cDNA.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 5 U of Poly(A) Polymerase incubated for 4 hours at 37 ℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 5 U of Poly(A) Polymerase incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 5 U of Poly(A) Polymerase incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

Using the third method of qPCR specified in General Chapter 3407 of ChP(2020) Volume IV, the residual *Escherichia coli* host cell DNA content of this product is below 10 copies/5 U.

Protocol

1. Prepare the following reaction mixture on ice:

Reagent	Amount
RNA[a]	1~10 μg
10× PAP Buffer	2 μl
Poly(A) Polymerase (5 U/μl)	1 μl
ATP (10 mM)	2 μl
RNase Inhibitor, Murine (40 U/μl) (Optional)	0.5~1 μl
Nuclease-Free Water	To 20 μl

a. Use purified RNA to prevent impurities from affecting poly(A) polymerase activity. If the 3' end of RNA has secondary structure, pre-denaturation of RNA is recommended (heat at 65℃ for 5 minutes, then place on ice for 5 minutes) prior to tailing to prevent impact on the tailing efficiency.

2. Incubate at 37℃ for 30~60 min. The tailing length is affected by multiple factors including enzyme amount, ATP concentration and reaction time. The reaction system can be adjusted according to actual results.

3. Using the reaction system described above, the length of the poly(A) tail can exceed 100 bases.

4. Add EDTA to a final concentration of 10 mM or incubate at 65℃ for 20 min to terminate the reaction.

Notice

1. Typically, this enzyme can add approximately 30 A bases during a 30-minute reaction at 37 ℃ and approximately 100 A bases during a 60-minute reaction.

2. This enzyme exhibits high selectivity in adding AMP to the 3' end of RNA and does not add Poly(A) tails of the same length to all RNA molecules.

3. After tailing, RNA must be purified prior to cell transfection or microinjection.

4. For your safety and health, please wear a lab coat and disposable gloves during the operation.

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