



Poly (A) Polymerase

Product	Quantity	Cat. No.	Remarks
Poly (A) Polymerase	50 unit	EBT-3002	1 unit/ μ l

Description

Poly A Polymerase catalyzes the addition of AMP to the 3' hydroxyl terminus of RNA. Poly A Polymerase can add poly(C) and poly(U) to RNA, but does so with much-reduced efficiency. Poly A Polymerase is purified from a recombinant *E.coli* strain.

Concentration & Storage Condition

1 unit/ μ l. Store at -20°C.

Storage Buffer

25 mM Tris-HCl, pH 7.9, 500 mM NaCl, 1 mM DTT, 1 mM EDTA and 50% (v/v) glycerol.

10x Reaction Buffer

500 mM Tris-HCl, pH 7.9, 2.5 M NaCl, 100 mM MgCl₂.

Unit Definition

One unit incorporates 1 nmole of AMP into tRNA in 10 min at 37°C.

QC Tests

Activity, exo and endonuclease activity test, SDS-PAGE purity, performance tests.

Usage information

1. Poly(A)-Tail Length:

The Poly(A) Tailing protocol will generate a poly(A)-tail length of ~150 b. However, the length of poly(A)-tail which can be synthesized by the A-Plus Poly(A) Polymerase is dependent upon several reaction parameters. Accordingly, users can customize poly(A)-tails to a desired length by adjusting one or more of these reaction parameters as outlined below.

Assuming all other reaction parameters are kept constant, poly(A)-tail length increases with:

- increasing units of A-Plus Poly(A) Polymerase (2-16 units).
- increasing time of incubation (10-60 min).
- decreasing amount of substrate RNA (60-1 mg).
- decreasing total reaction volume (100-10 μ l).

2. Poly(A)-Tailing Protocol

1) On ice, Add the following components to the microcentrifuge tube :

Target RNA	x μ l
10x Reaction Buffer	10 μ l
RNase inhibitor	1 μ l
10mM ATP	10 μ l
Poly(A) Polymerase	1-2 μ l
Nuclease-Free Water to final volume	100 μ l

2) Incubate at 37°C for 30 min. (Extending the incubation to 60 minutes results in poly(A)-tails > 200 b.)

3) The reaction may be stopped by any one of the following:

- a) immediate storage at -20°C.
- b) addition of EDTA to a final concentration of > 1 mM.
- c) phenol/chloroform extraction and salt/alcohol precipitation