DATA SHEET

Jena Bioscience

Poly(A) Tailing Enzyme Testkit

in vitro Polyadenylation of (m)RNA with *E. coli* and Yeast Poly(A) Polymerase

Cat. No.	Amount
RNT-004	75 reactions x 100 µl

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Description:
The Poly(A) Tailing Enzyme Testkit is designed to add a poly(A) tail to the 3' end of (m)RNA transcripts generated by *in vitro* transcription with HighYield T7 ARCA (m)RNA Synthesis Kits (#RNT-102, #RNT-103). The crude *in vitro* transcription reaction mix (approx. 10-30 µg RNA) can directly be used as template.

Polyadenylated (m)RNA exhibit increased stability and thus enhanced translation efficiency in transfection, microinjection or *in vitro* translation experiments in eukaryotic cells. The reaction is catalyzed by Poly(A) Polymerases that transfer AMP to 3'-hydroxyl ends of (m)RNA molecules. It is template-independent, requires ATP as substrate and Mg^{2+} or Mn^{2+} as cofactor.

The average length of attached poly(A) tails not only depends on RNA sequence, incubation time and enzyme concentration but also on the type of Poly(A) Polymerase. The Poly(A) Tailing Enzyme Testkit allows a direct comparison of *E. coli* and Yeast Poly(A) Polymerase to find the most suitable enzyme for a specific application.

The kit contains sufficient reagents for

- 25 reactions, 100 µl each with 20 units *E. coli* Poly(A) polymerase/reaction
- 50 reactions, 100 µl each with 600 units Yeast Poly(A) polymerase/reaction

Content:
***E. coli* Poly(A) Polymerase**
1x 100 µl (5 units/µl)
25 mM Tris-HCl, 500 mM NaCl, 1 mM $MgCl_2$, 0.1 mM DTT, 0.1 mM EDTA, 50% Glycerol (v/v), pH 8.0

***E. coli* Poly(A) Polymerase Reaction Buffer**
1x 1 ml (10x)
500 mM Tris-HCl (pH 8.0), 2.5 mM NaCl, 100 mM $MgCl_2$

***Yeast* Poly(A) Polymerase**
1x 50 µl (600 units/µl)
20 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.5 mM DTT, 50% Glycerol (v/v)

***Yeast* Poly(A) Polymerase Reaction Buffer**
1x 1.2 ml (5x)
100 mM Tris-HCl (pH 7.0), 3 mM $MnCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.5 mg/ml acetylated BSA, 50% glycerol (v/v)

ATP - Solution
1x 100 µl (100 mM)

PCR-grade water
5x 1.2 ml

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To be provided by user
RNA template (e.g. *in vitro* transcription reaction mix
RNA purification tools
RNAse-free DNAse I

1. Prevention of RNAse contamination

Although a potent RNase Inhibitor is included in most *in vitro* transcription reactions, creating a RNAse-free work environment and maintaining RNAse-free solutions is critical for performing successful polyadenylation reactions. We therefore recommend

- to perform all reactions in sterile, RNAse-free tubes using sterile pipette tips.
- to wear gloves when handling samples containing RNA.
- to keep all components tightly sealed both during storage and reaction procedure.

2. Poly(A) Tailing with *E. coli* Poly(A) Polymerase

The standard protocol typically generates poly(A) tails > 100 nt using 20 µl crude DNAse I-treated *in vitro* transcription reaction mix (approx. 10 - 30 µg, e.g. #RNT-102 or #RNT-103), however individual optimization of enzyme concentration and incubation time might be required. Please note: Do not stop DNAse I reaction with EDTA since EDTA interferes with the subsequent polyadenylation reaction.

Component	**Volume**	**Final conc.**
IVT reaction mix	19 µl	
PCR-grade water	X µl	
E. coli Poly(A) Polymerase Reaction Buffer (10x)	10 µl	1x
ATP (100 mM)	1 µl	1 mM
E. coli Poly(A) Polymerase (5 units/µl)	4 µl	20 units
Total volume	**100 µl**	

- Take 20 µl crude DNAse I-treated *in vitro* transcription reaction mix as template
- Safe 1 µl for poly(A) tailing efficiency analysis by agarose gel electrophoresis (negative control)
- Add PCR-grade water, *E. coli* Poly(A) Polymerase Reaction Buffer and ATP, voretex and spin-down briefly.
- Add *E. coli* Poly(A) Polymerase, voretex and spin-down briefly.
- Incubate for 60 min at 37°C (e.g. PCR cycler).
- Stop reaction by a) immediate freezing at -20°C or -70°C or b) immediate purification e.g. spin column-based or c) addition of EDTA to a final concentration > 11 mM.

3. Poly(A) Tailing with Yeast Poly(A) Polymerase

The standard protocol typically generates poly(A) tails > 100 nt using 20 µl crude DNAse I-treated *in vitro* transcription reaction mix (approx. 10 - 30 µg, e.g. #RNT-102 or #RNT-103), however individual optimization of enzyme concentration and incubation time might be required. Please note: Do not stop DNAse I reaction with EDTA since EDTA interferes with the subsequent polyadenylation reaction.

Component	**Volume**	**Final conc.**
IVT reaction mix	19 µl	
PCR-grade water	X µl	
Yeast Poly(A) Polymerase Reaction Buffer (5x)	20 µl	1x
ATP (100 mM)	1 µl	1 mM
Yeast Poly(A) Polymerase (600 units/µl)	1 µl	600 units
Total volume	**100 µl**	

- Take 20 µl crude DNAse I-treated *in vitro* transcription reaction mix as template
- Safe 1 µl for poly(A) tailing efficiency analysis by agarose gel electrophoresis (negative control)
- Add PCR-grade water, Yeast Poly(A) Polymerase Reaction Buffer and ATP, voretex and spin-down briefly.
- Add Yeast Poly(A) Polymerase, voretex and spin-down briefly.
- Incubate for 30 min at 37°C (e.g. PCR cycler).
- Stop reaction by a) immediate freezing at -20°C or -70°C or b) immediate purification e.g. spin column-based or c) addition of EDTA to a final concentration > 11 mM.

Please note: Reagents for the following steps are not provided within this kit.

Analysis of poly(A) tailing efficiency by agarose gel electrophoresis
Poly(A) tailed (m)RNA is longer than its untailed counterpart. This poly(A) tail-depending size shift can be visualized e.g. by agarose gel electrophoresis

- Pour a native TAE or denaturing formaldehyd agarose gel including ethidium bromide of the appropriate percentage (1% for RNA transcripts > 500 nt, 2% for RNA transcripts < 500 nt – 100 nt)
- Add a formamide-containing loading buffer to all RNA probes

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(before tailing (negative control) and after tailing reaction). The loading buffer must include > 20 mM EDTA to prevent heat degradation caused by divalent cations such as MgCl2 (e.g. 2x RNA Loading Dye, ThermoScientific).

- Incubate for 10 min at 70°C
- Load samples and run TAE agarose gel at approx. 5V/cm gel.
- Analyze poly(A) tailing efficiency by UV excitation.

(m)RNA purification
Purification of (m)RNA is required prior to some downstream applications e.g. spectroscopic quantitation or transfection. Spin column purification will remove proteins, salts and unincorporated nucleotides. Please follow the manufacturer instructions and ensure that the columns match with product size and possess a sufficient binding capacity ≥ 50 µg or ≥ 500 µg (e.g. RNA Clean & Concentrator™ columns (Zymo Research) or Monarch® RNA Cleanup kit (NEB)).

(m)RNA quantitation
(m)RNA concentration can be determined by absorbance measurement at 260 nm (A260) according to the Law-of-Lambert-Beer (A_{260} = 1 correspond to 40 µg/ml ssRNA).

Related Products:
E. coli Poly(A) Polymerase, #RNT-005
Yeast Poly(A) Polymerase, #RNT-006