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DATA SHEET

Version: 02
Revision date: 13/03/2023

1. Identification

Product name

CVX™ Poly(A) Tailing Kit
For Polyadenylation of RNA
50 reactions

Cat Nº **RS030**

2. Description

The Poly(A) Polymerase enzyme adds a chain of adenine nucleotides to the RNA. This process, called polyadenylation, increases the stability of RNA in eukaryotic cells and prevents its degradation.
The CVX Poly(A) Tailing kit is designed to add a poly(A) tail to the 3' end of (m)RNA transcripts generated by in vitro transcription with **CVX T7 ARCA (m)RNA Synthesis Kits**. Then the resulting capped and tailed RNA can be used in transfection or micro-injection experiments where mRNA stability and translation efficiency may be increased relative to unmodified mRNA..

3. Kit components

Item	Amount
E. coli Poly(A) Polymerase (5 units/µl)	100 µl
E. coli Poly(A) Polymerase Reaction Buffer (10X)	1 ml
ATP (100 mM)	100 µl
Nuclease-Free Water	5 ml

4. Features

- Quality Kit: High enzymatic purity, specificity, and activity
- Flexible: Use polyadenylated RNAs in a variety of applications.
- Fast and Easy: Simple protocol produces polyadenylated RNA molecules quickly.

5. Storage specifications

Store all kit components at -20 ºC. Avoid freeze/thaw cycles.
The CVX Poly(A) Tailing kit components are stable up to the expiry date specified with the product.

6. Applications

- Addition of a poly(A) tail to RNA synthesized *in vitro* in order to increase the stability.
- Addition of a poly(A) tail to an RNA molecule or a mixture of RNA molecules in order to provide priming site for synthesis of first-strand cDNA using a primer with poly(dT) on its 3´ end.
- Synthesis of polyadenylated RNA for nucleic acid amplification methods or gene expression studies..

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7. Further information

Product Use Limitations

This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer

The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

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Tailing protocol

Materials to Be Supplied by the User:

- RNA template (e.g. in vitro transcription reaction mix)
- RNA purification tools
- RNAse-free DNAse I

Important Notes before starting:

- Take all precautions to avoid RNase contamination during the whole process. We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination.
- All reagents should be spin briefly before opening to prevent loss and/or contamination.
- 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), however individual optimization of enzyme concentration and incubation time might be required.
- Do not stop DNAse I reaction with EDTA since EDTA interferes with the subsequentpolyadenylation reaction.

Transcription Reaction Setup (100 µL):

1. Start with a completed, 20 µl crude DNAse I-treated in vitro transcription reaction mix at room temperature. Do not add EDTA to the reaction as is sometimes recommended to inactivate DNase.
2. Safe 1 µl for poly(A) tailing efficiency analysis by agarose gel electrophoresis (negative control)
3. Assemble the reaction at room temperature in the following order.

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

4. Mix thoroughly, pulse-spin in microfuge. Incubate at 37˚C for 1 hours.
5. Place reaction on ice or store at –20˚C.