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DATA SHEET

Version: 02
Revision date: 13/03/2023

1. Identification

Product name **CVX T7 ARCA mRNA Synthesis Kit**
(50 reactions)

Cat. No RS018

2. Description

CVX T7 ARCA mRNA Synthesis Kit is designed for in vitro ARCA-capped (m)RNA Synthesis by T7 RNA Polymerase. The cap structure is added to the mRNA by co-transcriptional incorporation of Anti-Reverse Cap Analog (ARCA). ARCA is incorporated into mRNA exclusively in the correct orientation, generating capped mRNA that is more efficiently translated.

By using a DNA template encoding a poly(A) tail, the **CVX T7 ARCA mRNA Kit** can be used to synthesize capped and tailed mRNAs.

mRNAs synthesized with the **CVX T7 ARCA mRNA Kit** can subsequently be used for cell transfection, microinjection, in vitro translation and RNA vaccines.
The kit contains sufficient reagents for 50 reactions of 20 µl each. A 20 µl reaction yields about 50 µg RNA with 1 µg of template added (1.5 kb RNA transcript). The T7 RNA Polymerase Enzyme mix includes Ribonuclease Inhibitor Protein to protect newly transcribed RNA from degradation by RNases.

3. Composition

Item	Volume
T7 RNA Polymerase mix *	3 x 40 µl
T7 10X Reaction Buffer **	200 µl
ATP (100 mM)	100 µl
GTP (100 mM)	100 µl
CTP (100 mM)	100 µl
UTP (100 mM)	100 µl
DTT (100 mM)	100 µl
ARCA Solution (100mM)	10 µl
T7 Control template 2 (200 ng/µl)	10 µl
Nuclease-Free Water	1.2 ml

Notes:

*Include RNase inhibitor

**HEPES-based

4. Features

- High yield. Produce microgram amounts of RNA from all templates.
- Scaleable. Increase the reaction size to produce milligram amounts of RNA.
- Convenient. RNase inhibitor included.

5. Storage specifications

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Store all kit components at -20 °C. Avoid freeze/thaw cycles.
CVX SP6 Transcription Kit components are stable up to the expiry date specified with the product.

6. Applications

- In vitro transcription
- In vitro translation
- Hybridization probes generation
- RNase protection assays
- RNA binding protein assays
- Synthesis of antisense RNA

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 handeling or from contact with the above product.

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Before you start

The protocol is optimized for 0.5 µg - 1 µg DNA template

Materials to Be Supplied by the User:

- T7 Promotor-containing DNA template
- 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.
- Reactions are typically 20 µL but can be scaled up as needed.
- All reagents should be spin briefly before opening to prevent loss and/or contamination

IN VITRO TRANSCRIPTION PROTOCOL

1. Place T7 RNA Polymerase Mix on ice.

2. Thaw all remaining components at room temperature (RT), mix and pulse-spin in microfuge to collect solutions to bottom of tubes.

3. Assemble the reaction at room temperature in the following order:

Component	Volume	Final conc.
1. Nuclease-Free Water	X µl	
2. T7 10X Reaction Buffer (10x)	2 µl	1x
3. DTT (100 mM)	2 µl	10 mM
4. ARCA (100 mM)	1.2 µl	6 mM
5. GTP (100 mM)	0.3 µl	1.5 mM
6. UTP (100 mM)	1.5 µl	7.5 mM
7. CTP (100 mM)	1.5 µl	7.5 mM
8. ATP (100 mM)	1.5 µl	7.5 mM
9. Template DNA	X µl	1 µg
10. T7 RNA Polymerase Mix	2 µl	

4. Mix thoroughly, pulse-spin in microfuge. Incubate at 37°C for 2 hours.

5. **Optional:** Once the in vitro transcription reaction is finished, treat the sample with RNase-Free DNase I to remove template DNA and purify the RNA. For many applications it may not be necessary. Proceed with a DNase digestion as following:

   Add RNase-free DNase I (not provided) plus the respective reaction buffer directly to the transcription reaction.

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Mix and incubate for 15 minutes at 37 °C.
Stop DNase activity by adding 1 µL of EDTA at 0.5 M.

6. Proceed with purification of synthesized RNA or analysis of transcription products by gel electrophoresis.