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DATA SHEET

Version: 02
Revision date: 13/03/2023

1. Identification

Product name **CVX T7 RNA Synthesis Kit**
50 reactions

Cat. No **RS012**

2. Description

CVX T7 Transcription Kit is designed for in vitro RNA synthesis by T7 RNA Polymerase from linearized plasmid DNA templates or DNA templates generated by PCR. Synthesized RNA can subsequently be used for in vitro translation, RNase protection assays, RNA splicing, hybridization assays and Northern Blot experiments.
The kit contains sufficient reagents for 50 reactions of 20 µl each. A 20 µl reaction yields about 150 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
T7 Control template 1 (200 ng/µl)	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

Store all kit components at -20 °C. Avoid freeze/thaw cycles.
CVX T7 RNA Synthesis 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

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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 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:

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