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MiniV™ *In Vitro* Transcription Kit

Cat. No. MV41025

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1. Introduction

The MiniV™ *In Vitro* Transcription Kit provides all of the components necessary for *in vitro* transcription of RNA from single-stranded DNA (ssDNA) templates. MiniV RNA Polymerase* is a transcriptionally-active 1,106 amino acid domain of the N4 virion RNA polymerase capable of transcribing ssDNA templates containing an N4 promoter sequence.

Since the enzyme lacks RNA strand displacement or unwinding activity on RNA:DNA hybrids, MiniV RNA Polymerase requires *E. coli* Single-Stranded DNA Binding Protein (EcoSSB) to displace the RNA strand from the DNA template for efficient *in vitro* transcription. The yield of RNA from a MiniV transcription reaction varies depending on the sequence of the DNA template. Typically 1-5 moles of RNA are produced per mole of ssDNA template.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle. Do not store at –70°C.

Contaminating Activity Assays: All of the components of the MiniV *In Vitro* Transcription Kit are free of detectable RNase activity, and all of the components except DNase I are free of detectable exo- and endonuclease activities.

MiniV Control ssDNA Template: The Control ssDNA Template provided in the kit is a 71 base oligodeoxynucleotide containing the MiniV N4 P2 transcription promoter near its 3'-end. Transcription of the control template produces a 43 base RNA transcript. The complete sequence of the Control Template is provided in section 2, page 2.

DNase I Unit Definition:1 Molecular Biology Unit (MBU) of DNase I digests 1 microgram of pUC19 DNA to oligodeoxynucleotides in 10 minutes at 37°C.

3. Kit Contents

Desc.	Concentration	Quantity
The MiniV *In Vitro* Transcription Kit is available in a 25-reaction size containing:		
MiniV™ RNA Polymerase	(100 pmol/µl)	
(contains an RNase inhibitor)		25 µl
MiniV™ 5X Transcription Buffer		100 µl
Single-Stranded DNA Binding Protein, *E. coli*	(1 µg/µl; 53 pmol/µl)	30 µl
10 mM ATP, CTP, GTP and UTP Solutions		each at 40 µl
20 mM Dithiothreitol (DTT)		25 µl
RNase-Free Water		500 µl
MiniV™ Control ssDNA Template (2 pmol/µl)		15 µl
RNase-Free DNase I	@ 1 MBU/µl	25 µl

4. Related Products

The following products are also available:

– *E. coli* Single-Stranded DNA Binding Protein
– NTP Solutions

5. Notes on Using the MiniV *In Vitro* Transcription Kit

1. **MiniV Promoter Sequences:** Three functional phage N4 transcription promoters have been characterized.[1]

   P1: 5′ - CCATAAGTTGCGAAGCAAC - 3′

   P2: 5′ - CCAAAAGAAGCGGAGCTTCTT - 3′

   P3: 5′ - CCAAAAGCTGCGGAGCAGC - 3′

Promoter P2 has produced the best results at Epicentre. Therefore, we recommend incorporating the P2 promoter into user-designed ssDNA templates.

2. **Designing a MiniV ssDNA Transcription Template:** The ssDNA transcription template is transcribed in the 3′→5′ direction, generating RNA in the 5′→3′ direction. Therefore, the MiniV transcription promoter should be at or very near the 3′-end of the ssDNA template. The Control ssDNA Template provided in the MiniV *In Vitro* Transcription Kit provides an example (MiniV N4 P2 Promoter is shown in ***bold italic***):

MiniV Control ssDNA Template (71-bases)

5′- CAAGATCATTGCTCCTCCTGAGCGCAAGTACTCCGTGTGGA
CCAAAAGAAGCGGAGCTTCTTAATGAAGAT -3′

| (+1)

←...ACACCUGG -5′ RNA Transcript

3. **Purity of a Synthetic Single-stranded Oligodeoxynucleotide Template:** Inefficiencies inherent in chemical synthesis of oligodeoxynucleotides will cause an oligo to be contaminated by prematurely truncated synthesis products. If these premature termination products contain an N4 Polymerase promoter, they may be transcribed in a MiniV transcription reaction resulting in the production of multiple RNA transcripts from a single reaction.

   To better ensure that only the desired RNA species is transcribed in the MiniV transcription reaction, it may be necessary for the user or the oligodeoxynucleotide provider to purify the oligo template by HPLC or gel electrophoresis prior to the transcription reaction. Though this will not eliminate all premature synthesis products, it will improve the proportion of the desired RNA transcript.

4. **Converting Micrograms of an Oligonucleotide Template to Picomoles:** The standard MiniV transcription reaction uses 10 pmol of a single-stranded DNA template. To convert micrograms of an oligonucleotide to picomoles, use the equation:

   (x µg of oligonucleotide) x (10^6) / ([number of nucleotides] x 330) = pmol of oligonucleotide

   Example: 1 µg of a 50 base oligonucleotide = ? pmol of oligonucleotide

   1 x 10^6 / ([50] x 330) = 60.6 pmol

5. **Requirement for E. coli Single-Strand DNA Binding Protein (EcoSSB):** MiniV RNA Polymerase is unable to "unwind" RNA:DNA hybrids. Therefore, when producing RNA transcripts of >24 bases, the MiniV transcription reaction requires EcoSSB to displace the RNA transcript from the DNA template strand for efficient *in vitro* transcription. Experiments at Epicentre indicate that EcoSSB does not enhance the transcription efficiency of RNA transcripts <24 bases in length.

   The standard MiniV transcription reaction uses an optimal 5:1 molar ratio of EcoSSB to ssDNA template. The standard MiniV transcription reaction contains 1 µg of EcoSSB (53 pmol; MW = 18,884) and 10 pmol of ssDNA template. Increasing the amount of template can increase the yield of RNA. Therefore, for every additional 5 pmol of template added to the reaction, be sure to add an additional 0.5 µl of the 1 µg/µl EcoSSB to the reaction.

6. MiniV *In Vitro* Transcription Procedure

1. Combine the following reaction components on ice.

			Final Concentration
x	µl	RNase-Free water	---
10	pmol	Single-stranded DNA template with appropriate promoter **	0.5 pmol/µl
4	µl	MiniV 5X Transcription Buffer	1X
1.5	µl	10 mM ATP	0.75 mM
1.5	µl	10 mM CTP	0.75 mM
1.5	µl	10 mM GTP	0.75 mM
1.5	µl	10 mM UTP	0.75 mM
1	µl	20 mM DTT	1 mM
1	µl	1 µg/µl (53 pmol/µl) *E. coli* SSB Protein	50 ng/µl
1	µl	(100 pmol/µl) MiniV N4 RNA Polymerase	5 pmol/µl
20	µl	Total reaction volume	

2. Incubate at 37°C for 30 minutes.
3. Optional: Treat the sample with RNase-Free DNase I to remove DNA template and purify the RNA as described on page 5.

***See page 3 for further information.*

DNase I Treatment

If removal of the ssDNA template is desired, digest with RNase-Free DNase I.

1. Add 1 µl (1 MBU) of RNase-Free DNase I to the reaction and incubate for 15 minutes at 37°C.
2. Heat the reaction for 15 minutes at 70°C to inactivate the DNase. Proceed to "Purification of the RNA" below.

Purification of the RNA

Three methods for purifying the RNA transcript are presented below. Choose the method which best suits the intended subsequent use of the RNA. Spin chromatography yields the cleanest RNA. Ammonium acetate precipitation and ethanol precipitation selectively precipitates RNA while by leaving much (but not all) of the DNA, protein and unincorporated NTPs in the supernatant.

Spin chromatography: This method is applicable to all sizes of RNA transcripts.

1. Epicentre has successfully used Micro Bio-Spin® Columns with Bio-Gel P-6 (Bio-Rad) in Tris Buffer and MicroSpin™ G-25 columns (Amersham) to purify RNA from an *in vitro* transcription reaction.

Ammonium acetate precipitation: This method is applicable for RNA transcripts >100 bases in size.

1. Add 1 volume of 5 M ammonium acetate (20-21 µl for the standard MiniV transcription reaction).
2. Incubate on ice for 10-15 minutes.
3. Centrifuge at high speed (e.g. 10,000 x g) for 10-15 minutes at room temperature or 4°C.
4. Remove the supernatant carefully with a pipette and gently rinse the pellet with 70% ethanol.
5. RNA can be stored at –20°C or –70°C as a dry pellet or resuspended in RNase-Free water, $T_{10}E_1$ or other suitable buffer.
6. Optional: Repeat Steps 1-5 to remove residual contaminants.

Ethanol precipitation: This method is applicable to all sizes of RNA transcripts.

1. Add sodium acetate to a final concentration of 0.3 M, followed by 2.5 volumes of ethanol.
2. Incubate at –20°C for 30 minutes.
3. Centrifuge at high speed (e.g. 10,000 x g) for 10-15 minutes at room temperature or 4°C.
4. Remove the supernatant carefully with a pipette and gently rinse the pellet with 70% ethanol.
5. RNA can be stored at –20°C or –70°C as an ethanol pellet, dry pellet or resuspended in RNase-Free water, $T_{10}E_1$ or other suitable buffer.

7. Reference

1. Kazmierczak, K.M. *et al.*, (2002) *EMBO J.* **21**, 5815.

General References:

2. Haynes, L.L. and Rothman-Denes L.B. (1985) *Cell* **41**, 597.
3. Glucksmann, M.A. *et al.*, (1992) *Cell* **70**, 491.
4. Schwarz, K. *et al.*, (1990) *Nucl. Acids Res.* **18**, 1079.
5. Davydova, E.K. and Rothman-Denes L.B. (2003) *ProC. Natl. Acad. Sci. USA* **100**, 9250.

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