

Background

Bacteriophage T7 RNA Polymerase is a 99 kDa protein that recognizes T7 phage promoters with high specificity and subsequently initiates transcription. T7 RNA polymerase is a single subunit, highly processive and stable enzyme, characteristics that make it suitable for a broad range of biochemistry and molecular biology applications.

Description

The Aurora T7 RNA Polymerase In Vitro Transcription Kit is a quick and easy approach to generate large amounts of RNA in vitro. The RNA product from the kit is suitable for RNA structural, functional, and enzymatic (ie. ribozyme) studies, production of RNA probes for hybridization blotting or RNase protection assays, RNA vaccine production, microarray and microinjection, anti-sense RNA and RNAi experiments, and in vitro translation.

The assay is fast and convenient, and requires the T7 RNA polymerase, NTP mix (UTP, ATP, CTP, and GTP), reaction buffer, and a suitable DNA template. The modified nucleotide N1-Methyl-Pseudo UTP is incorporated in our T7 RNA Polymerase In Vitro Transcription Kit-II.

Figure 1 illustrates the T7 transcription with T7 promoter sequence and the transcription start site.

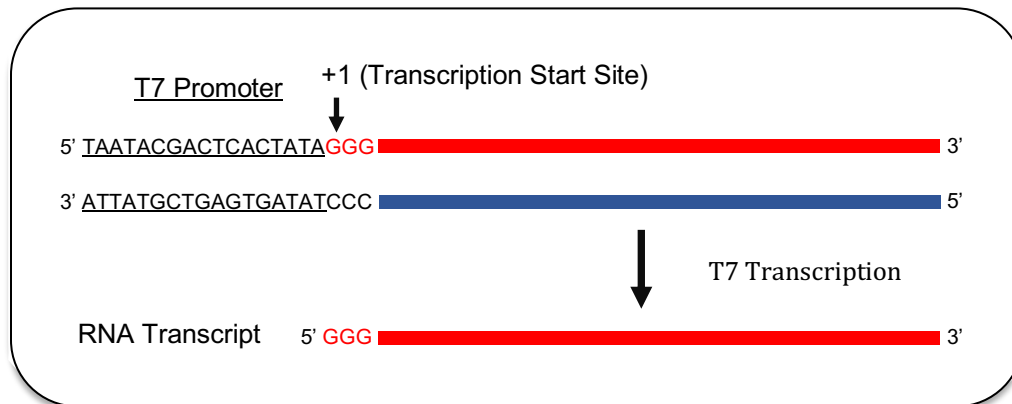


Figure 1. T7 RNA transcription

Materials Supplied

Catalogue No.	Components	25 rxns	50 rxns	100 rxns
K777627-E	T7 RNA Polymerase MIX	50 µL	100 µL	200 µL
K777627-B	10X Reaction Buffer	50 µL	100 µL	200 µL
K777627-A	ATP (20 mM)	50 µL	100 µL	200 µL
K777627-G	GTP (20 mM)	50 µL	100 µL	200 µL
K777627-C	CTP (20 mM)	50 µL	100 µL	200 µL
K777627-U	UTP (20 mM)	50 µL	100 µL	200 µL
K777627-T	Control Template (0.5 ug/µL)	5 µL	10 µL	20 µL
K777627-H	RNase-free H ₂ O	0.5 ml	1 ml	2X1 ml

Store all the kit components at -20C.

Stability

12 months if stored under the indicated conditions.

Assay Protocol

1. DNA template preparation

Plasmid DNA, PCR DNA products or synthetic DNA oligonucleotides can be used as templates for in vitro transcription with the T7 RNA polymerase in this Kit. Double-stranded T7 promoter sequence must be located upstream of the sequence to be transcribed. Plasmid DNA vector should be linearized by the restriction enzyme(s) and purified before transcription.

2. Set up reactions for RNA synthesis

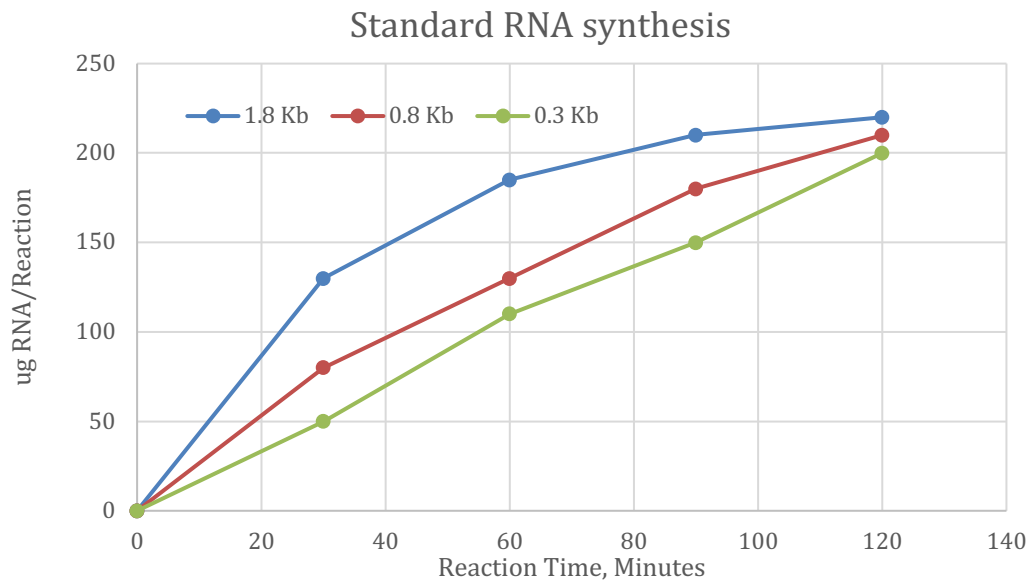
- 1) Thaw the kit components on ice and briefly spin the tubes to recover the full contents at the bottom of the tube. For T7 RNA polymerase, make aliquots of the enzyme for single use. Store remaining aliquot protein at -80°C.
- 2) Assemble the reaction at room temperature in the following order:

Protocol Summary

Component	Working Solution	Stock Solution	Vol of Stock (µl)
Nuclease-free water	X µl		X µl
10X Reaction Buffer	1X	10X	2 µl
ATP (20 mM)	2 mM	20 mM	2 µl
GTP (20 mM)	2 mM	20 mM	2 µl
CTP (20 mM)	2 mM	20 mM	2 µl
UTP (20 mM)	2 mM	20 mM	2 µl
Template DNA	0.2-1 ug		X µl
T7 RNA polymerase	10 mM		2 µl
Total Volume			20 µl

- 3) If you want to run multiple reactions with different templates, you can prepare a master mix containing the 10X reaction buffer and four ribonucleotide (NTP) solutions. Use 10 µl per reaction. Prepare a little more master solution to make sure it is enough for the reactions. Then, add the template and T7 RNA polymerase separately.
- 4) Mix thoroughly with the pipette, pulse-spin in microfuge.
- 5) Incubate at 37°C for 2 hours. Incubate the transcripts of short fragments (<300nt) for 4h. The yield will not be compromised if the incubation temperature is within the range of 35–40°C.

Data Analysis



Related products:

Catalog #	Product Name	Size
5727-4121NK	Kras WT Nucleotide Exchange Assay Kit	384 reactions
5727-4122NK	Kras G12C Nucleotide Exchange Assay Kit	384 reactions
5727-4123NK	Kras G12D Nucleotide Exchange Assay Kit	384 reactions
5727-4133NK	Kras G13D Nucleotide Exchange Assay Kit	384 reactions
5727-4127NK	Kras G12R Nucleotide Exchange Assay Kit	384 reactions
5727-4128NK	Kras G12V Nucleotide Exchange Assay Kit	384 reactions
5727-4121BK	Kras WT-cRAF Binding Assay Kit	384 reactions
5727-4122BK	Kras G12C-cRAF Binding Assay Kit	384 reactions
5727-4123BK	Kras G12D-cRAF Binding Assay Kit	384 reactions
5727-4127BK	Kras G12R-cRAF Binding Assay Kit	384 reactions
5727-4128BK	Kras G12V-cRAF Binding Assay Kit	384 reactions
728203	SARS-CoV-2 Mpro (3CL Protease) Assay Kit	96 reactions
728253	SARS-CoV-2 Papain-like Protease Assay Kit	96 reactions
728263	SARS-CoV-2 Nucleocapsid Protein Binding Kit (For mouse antibody)	384 reactions
728273	SARS-CoV-2 Nucleocapsid Protein Binding Kit (For rabbit antibody)	384 reactions
362101	DNA Polymerase Theta Activity Assay Kit	96 reactions, 384 rec
K777627	T7 High Yield RNA Synthesis Kit	25, 50, 100 reactions
756981BK	PKMYT1 Binding Assay Kit	384 reactions
759331BK	WEE1 Binding Assay Kit	384 reactions
34343BK	eIF4E/eIF4G Binding Assay Kit	384 reactions
810030	Caspase-3 Activity Assay Kit	384 reactions
910010	IDO1 Activity Assay Kit for Inhibitor Screening	96 reactions

5727-4122G	Kras G12C, GST-tag	50 µg, 100 µg
5727-4123G-G	Kras G12D, GST-tag, GDP Loaded	50 µg, 100 µg
5727-4128G-GP	Kras G12V, GST-tag, GppNHP loaded	50 µg, 100 µg
7671	SOS1, No tag	100 µg, 1 mg
7671HA	SOS1, His-Avi-tagged	100 µg, 1 mg
7237231	Human RBD-RAF1, N-His tag, C-FLAG tag	100 µg
180001	Recombinant Human Malic enzyme 1 (ME1)	10 µg, 100 µg, 500 µg, 1 mg
180002	Recombinant Human Malic enzyme 2 (ME2)	10 µg, 100 µg, 500 µg, 1 mg
180003	Recombinant Human Malic enzyme 3 (ME3)	10 µg, 100 µg, 500 µg, 1 mg
12-0009MH	Recombinant His-tagged Human MNK2 D228G	10 µg, 100 µg
12-0009H	Recombinant His-tagged Human MNK2	10 µg, 100 µg
12-0009M	Recombinant Human MNK2 D228G	10 µg, 100 µg
12-0009	Recombinant Human MNK2	10 µg, 100 µg
12-0010	His-tagged Human eIF4E	50 µg, 100 µg
12-0011	His-tagged Human eIF4E Complexed With EIF4G	50 µg, 100 µg
12-0012	His-tagged Human eIF4E Complexed With m7GTP	50 µg, 100 µg
7657643	DNA Polymerase Theta-N-Helicase Domain	20 µg, 100 µg
7657283	DNA Polymerase Theta-C terminal Domain	20 µg, 100 µg
C352E1-10	GST-CDK2:his-CyclinE1	10 µg
C352A2-10	GST-CDK2:His-CyclinA2	10 µg
225201-1	Recombinant Human BCL2	100 µg
62581	Human MALT1 (caspase-IG3)	20 µg
232340	Recombinant Human CD40	100 µg
2323405	Recombinant Human CD40L	100 µg
2323155	Recombinant Human CD155	100 µg
236940	Recombinant Human OX40	100 µg
2369405	Recombinant Human OX40L	100 µg
2344875	Recombinant Human GITRL	100 µg
237351	Recombinant Human PD-L1	100 µg
56781	Recombinant Full-length Human MST1	10 µg, 50 µg, 100 µg, 500 µg
52352-FL	Recombinant Human CDK2	50 µg, 500 µg
5756981-FL	Recombinant Human Full Length PKMYT1	10 µg, 50 µg, 100 µg, 500 µg
5756981-CDD	Recombinant Human PKMYT1, catalytic domain – dephosphorylated	10 µg, 50 µg, 100 µg, 500 µg
5756981-CDP	Recombinant Human PKMYT1, catalytic domain – phosphorylated	10 µg, 50 µg, 100 µg, 500 µg

Products are for research use only and are not intended for human use. We do not sell to patients.