T7 High Yield RNA Synthesis Kit

Catalog Number: K777627

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.

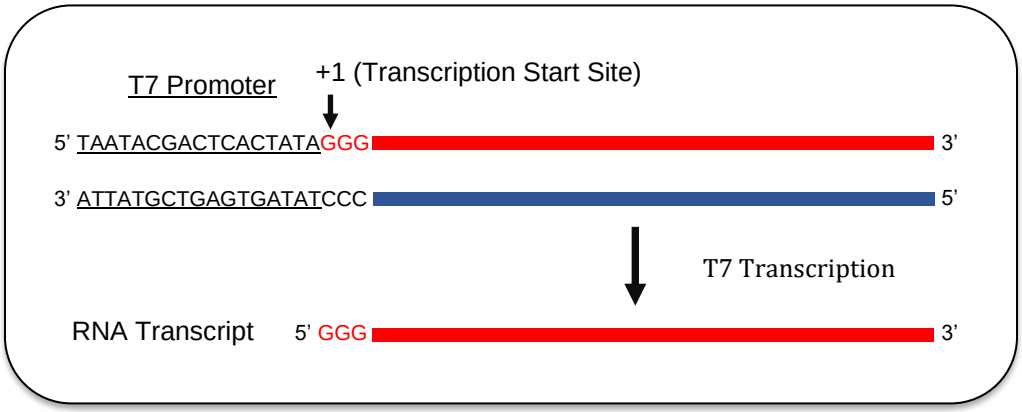


Figure 1. T7 RNA transcription

Materials Supplied

Components	25 rxns	50 rxns	100 rxns
T7 RNA Polymerase MIX	50 µL	100 µL	200 µL
10X Reaction Buffer	50 µL	100 µL	200 µL
ATP (20 mM)	50 µL	100 µL	200 µL
GTP (20 mM)	50 µL	100 µL	200 µL
CTP (20 mM)	50 µL	100 µL	200 µL
UTP (20 mM)	50 µL	100 µL	200 µL
Control Template (0.5 ug/µL)	5 µL	10 µL	20 µL
RNase-free H_2O	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

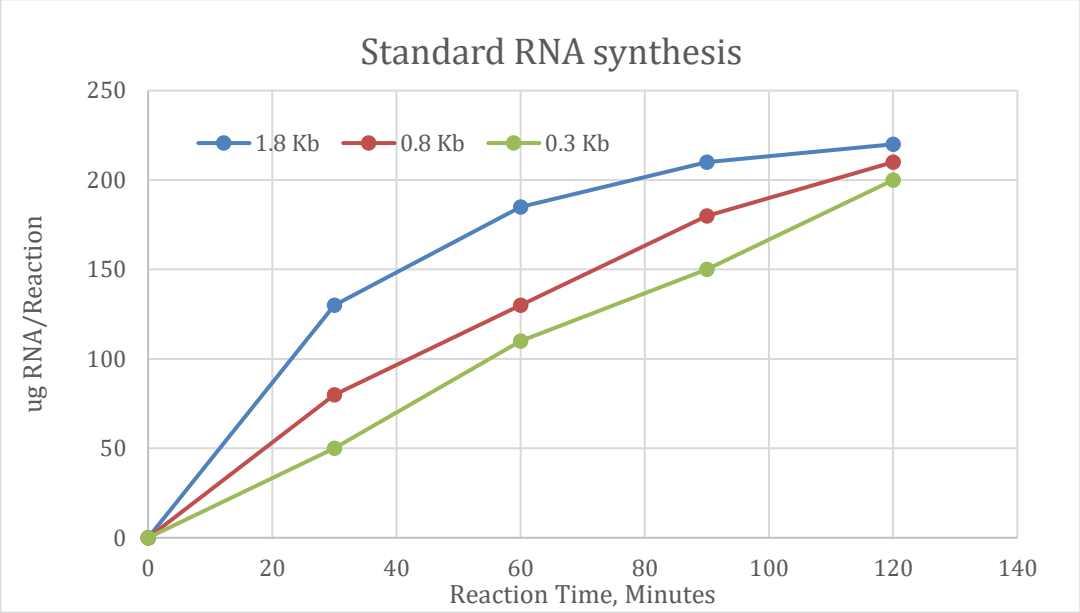
Component	Working Solution	Stock Solution	Vol of Stock (µl)
Nuclease-free water	X µl		X µl
10X Reaction Buffer	1X	10X	2 µl
ATP (20 mM)	2 mM	20 mM	2 µl
GTP (20 mM)	2 mM	20 mM	2 µl
CTP (20 mM)	2 mM	20 mM	2 µl
UTP (20 mM)	2 mM	20 mM	2 µl
Template DNA	0.2-1 ug		X µl
T7 RNA polymerase	10 mM		2 µl
Total Volume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



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