Aurora Biolabs
High Purity Protein for Research

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.

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.

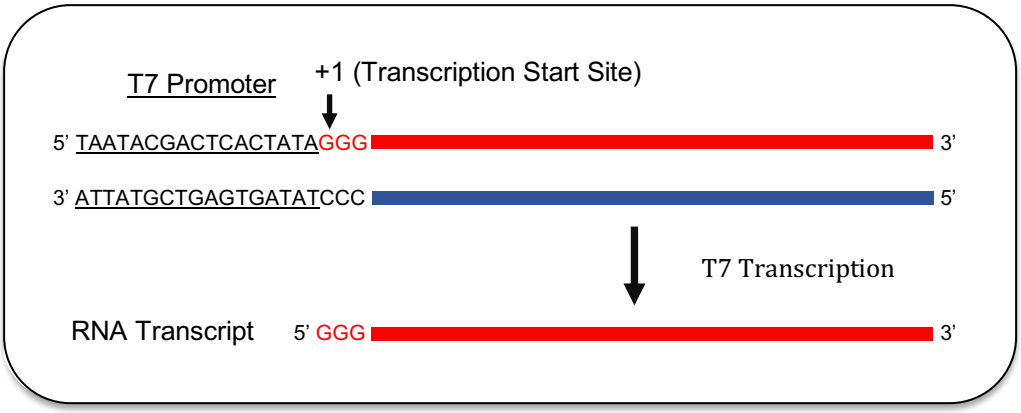


Figure 1. T7 RNA transcription

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.

RNA Synthesis Protocol

1. DNA template preparation

   Plasmid DNA, DNA PCR products and synthetic DNA oligonucleotides can be used as templates for in vitro transcription.

   1) Plasmid DNA

      Plasmid DNA with the T7 promoter should be linearized by the restriction enzyme(s) and purified before transcription. After enzyme digestion, the linearized DNA can be purified by phenol/chloroform extraction or by DNA purification columns.

   2) PCR DNA products

      PCR products containing T7 promoter upstream the coding sequence can be used as a template. The PCR product mixture can be directly used for transcription, but PCR products purified with RNA purification column will have better yield.

   3) Synthetic DNA oligonucleotides

      Two single stranded DNA oligonucleotides with complementary sequence carrying the T7 promoter can be used for the transcription. The DNA template is formed by annealing the two oligos.

2. Set up in vitro 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.

6) DNA template can be digested by adding 1 µl of DNase I to the reaction and incubate at 37°C for 15 minutes.

Purification of the Product

1. Phenol/chloroform Purification

   1) Add 160 µl of RNase-free ddH_2O to adjust the volume to 180 µl.

   2) Add 20 µl of 3M sodium acetate (pH5.2) or 20 µl of 5M ammonium acetate, and mix well with a pipette.

   3) Add equal volume (200 µl) of phenol/chloroform mixture (1:1), mix well and centrifuge at 12,000 rpm for 5 minutes at 4°C.

   4) Transfer the upper phase (aqueous phase) to a new tube, add 200 µl chloroform and extract twice with chloroform.

   5) Add two volumes of ethanol, mix well to precipitate RNA. Incubate at -20°C for at least 30 minutes, and then centrifuge at 12,000 rpm.

   6) Remove the supernatant and wash the pellet with 500 µl of 70% ethanol.

   7) Resuspend the RNA pellet with 20 – 50 µl of 0.01 mM EGTA. Store the RNA at -80°C.

2. Column Purification

   RNA purification columns are commercially available. The columns can remove unincorporated nucleotides and proteins.
   Dilute the RNA product to 100 µl by adding 80 µl of nuclease-free ddH_2O. Purify the RNA according to manufacturer's instruction.

Quantification of the Product

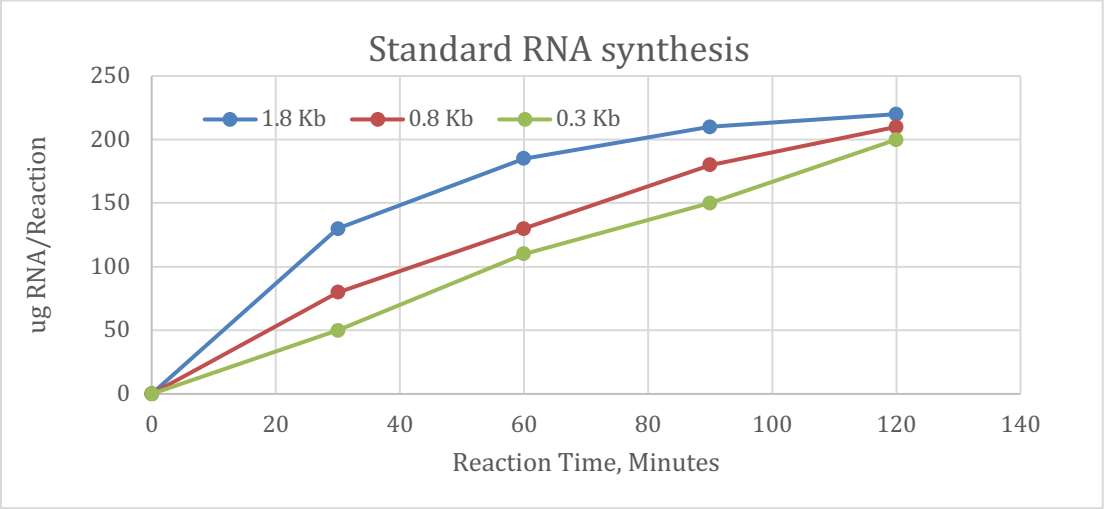
1. UV Light Quantification

   RNA concentration can be determined by measuring absorbance at 260 nm using a UV spectrophotometer. RNA samples should be purified after synthesis since free nucleotides and DNA template in the samples may affect the reading. Pure RNA samples have an absorbance ratio at 260 nm/280 nm of 2.0. One absorbance reading at 260 nm is equivalent to 40 µg/mL of RNA.

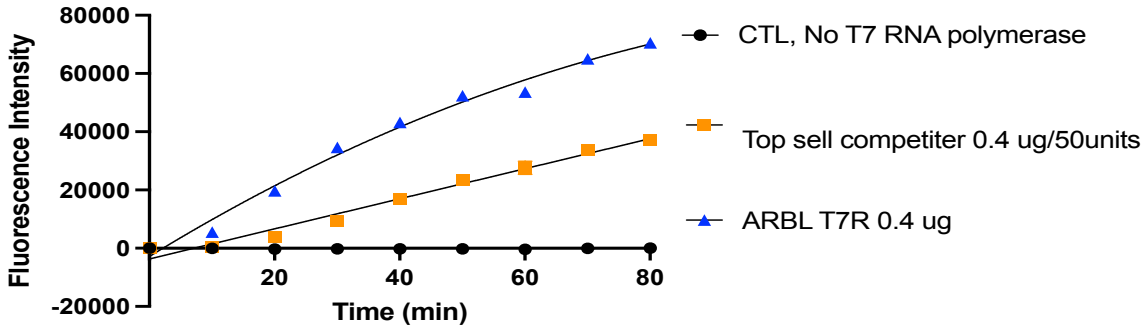
2. NanoDrop Quantification

   RNA concentration can be measured using a NanoDrop spectrophotometer.

Data Reference



T7 RNA plymerase Activity



Related products:

Product Name	Catalog #	Size
T7 RNA polymerase	777627	5000 U, 25000U, 100000U
T7 High Yield RNA Synthesis Kit	777627-RK	25 rxns, 50 rxns, 100 rxns
DNA Polymerase Theta Activity Assay Kit	362101	96 reactions
Kras Wild Type (WT), GST-tag	5727-4121G	50 µg, 100 µg
Kras WT, GST-tag, GDP Loaded	5727-WTG-G	50 µg, 100 µg
Kras WT, GST-tag, GppNHp loaded	5727-WTG-GP	50 µg, 100 µg
Kras G12C, His-tag	5727-4122H	50 µg, 100 µg
Kras G12C, GST-tag	5727-4122G	50 µg, 100 µg
Kras G12C, GST-tag, GDP Loaded	5727-4122G -G	50 µg, 100 µg

Kras G12C, GST-tag, GppNHp loaded	5727-4122G -GP	50 µg, 100 µg
Kras G12D, GST-tag	5727-4123G	50 µg, 100 µg
Kras G12D, GST-tag, GDP Loaded	5727-4123G -G	50 µg, 100 µg
Kras G12D, GST-tag, GppNHp loaded	5727-4123G -GP	50 µg, 100 µg
Kras G12R, GST-tag,	5727-4127G	50 µg, 100 µg
Kras G12R, GST-tag, GDP Loaded	5727-4127G -G	50 µg, 100 µg
Kras G12R, GST-tag, GppNHp loaded	5727-4127G -GP	50 µg, 100 µg
Kras G12V, GST-tag,	5727-4128G	50 µg, 100 µg
Kras G12V, GST-tag, GDP Loaded	5727-4128G -G	50 µg, 100 µg
Kras G12V, GST-tag, GppNHp loaded	5727-4128G -GP	50 µg, 100 µg
Kras WT Nucleotide Exchange Assay Kit	5727-4121NK	384 reactions
Kras G12C Nucleotide Exchange Assay Kit	5727-4122NK	384 reactions
Kras G12D Nucleotide Exchange Assay Kit	5727-4123NK	384 reactions
Kras G12R Nucleotide Exchange Assay Kit	5727-4127NK	384 reactions
Kras G12V Nucleotide Exchange Assay Kit	5727-4128NK	384 reactions
Kras WT – cRAF Binding Assay Kit	5727-4121BK	384 reactions
Kras G12C – cRAF Binding Assay Kit	5727-4122BK	384 reactions
Kras G12D– cRAF Binding Assay Kit	5727-4123BK	384 reactions
Kras G12R – cRAF Binding Assay Kit	5727-4127BK	384 reactions
Kras G12V – cRAF Binding Assay Kit	5727-4128BK	384 reactions
Human RBD-RAF1, N-His tag, C-FLAG tag	7237231	50 µg, 100 µg
Human SOS1, No Tag	7671	50 µg, 100 µg
Human SOS1, His-Avi-Tag	7671HA	50 µg, 100 µg
Recombinant SARS-CoV-2 Mpro, 3CL protease	728201	50 ug, 500 ug
SARS-CoV-2 Mpro (3CL Protease) Assay Kit	728203	96 reactions
Recombinant SARS-CoV-2 Papain-like Protease (PLpro, NSP3), CF	728251	50 ug, 100 ug, 1mg
Papain-like (PLpro) Protease Assay Kit	728253	96 reactions
Recombinant SARS-CoV-2 Helicase (NSP13)	728231	10 ug, 50 ug, 100 ug
Recombinant SARS-CoV-2 NSP7	728264	100 ug, 1mg

Recombinant SARS-CoV-2 NSP8	728265	100 ug, 1mg
TEV-His	190001-R	1,000 Units, 10,000 Units
Recombinant SUMO Protease (Ulp1)	190003	1,000 units, 10,000 units
PreScission Protease (HRV 3C)	190002-R	1,000 units, 10,000 units
Recombinant YopH	200100	10 ug, 20 ug, 100 ug, 1 mg
Recombinant Mouse Leukemia Inhibitory Factor (mLIF)	11-0001	10 ug, 100 ug
Recombinant Human LIF	12-0002	10 ug, 100 ug, 1 mg
Recombinant Human LIF, Animal-Free	12-0002AFR	10 ug, 100 ug, 1 mg
Recombinant Human FGF-basic, Carrier-free	12-0005CFR	50 ug, 100 ug, 500 ug, 1 mg
Recombinant Human BCL2	225201	100 ug
GST-CDK2: His-CyclinE1	C352E1	10 ug, 100 ug
GST-CDK2: His-CyclinA2	C352A2	10 ug, 100 ug
Recombinant Human Malic enzyme 1 (ME1)	180001	10 ug, 25 ug, 100 ug, 1 mg
Recombinant Human Malic enzyme 2 (ME2)	180002	10 ug, 25 ug, 100 ug, 1 mg
Recombinant Human Malic enzyme 3 (ME3)	180003	10 ug, 25 ug, 100 ug, 1 mg

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