Gene to Protein Pvt. Ltd.
www.genetoprotein.com
info@genetoprotein.com
800 GENOME, 800 GENETIC

In-vitro Transcription Kit (IVT)

Cat# ☐RNA010-25 ☐ RNA010-25-S
Pack Size: ☐ 25 Rxn ; ☐ 5 Rxn

Storage: -20°C

Introduction

This kit is design for in vitro transcription of RNA using T7 RNA polymerase. This standard 2hr incubation of 20µl reaction yields ~150µg of RNA from 1µg of DNA template. RNA synthesizes using this kit has a wide range of application like microarray analysis, Ribozyme biochemistry, in Vitro translation, RNA probes, RNA vaccines, Synthesis of capped RNA etc. This kit is may use be used in radiolabeled RNA probes synthesis. This kit is able to produce short RNA transcript as well as long transcript. The standard kit contains sufficient reagents for 50 reactions of 20µL each.

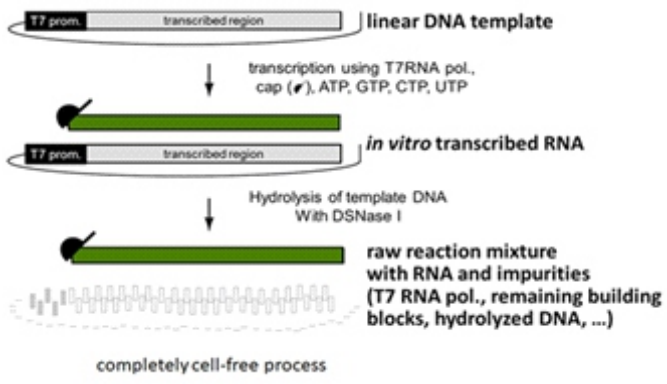
Kit content:

Components	RNA010-25	RNA010-25-S
RNase free water	1 mL	1mL
Template DNA	15µL (0.4µg/µL)	3µL (0.4µg/µL)
10X transcription Buffer	50µL	10µL
100mM ATP	38 µL	7.6 µL
100mM CTP	38 µL	7.6 µL
100mM GTP	38 µL	7.6 µL
100mM UTP	38 µL	7.6 µL
100mm DTT	50 µL	10 µL
RNase inhibitor	12.5 µL	2.5 µL
T7 RNA polymerase	50 µL	10 µL

DNA template Preparation:

Circular plasmid, linearized plasmid DNA, PCR amplified products or synthetic DNA oligonucleotide can be used as template for in vitro transcription using this kit provided that the upstream region of template contains T7 promoter sequence.

Figure1: Transcription by T7 RNA polymerase



Plasmid templates

Templates for in vitro transcription can be prepare by linearizing the circular plasmid by restriction endonucleases, Blunt end linearization is less preferable whereas templates with 5' protruding ends are more preferable.

Quality of plasmid template matters most for the reaction, highest purity plasmid can be achieved using standard kits containing no impurities like RNA, proteins, RNase and salts. Linearized plasmid before use should be purified. It is use in the range of 0.5-1µg concentration in a 20µl.

PCR templates

PCR amplified products are good templates for transcription, upstream sequence of target gene should contain T7 promoter region. PCR templates should be examined on agarose gel before use to see expected amplicon size and for better RNA yield it should be purified.

For purification there are many methods like Phenol: Chloroform precipitation or by using commercially available spin column. PCR templates in the range of 0.5-1 µg concentration in a 20µl can be use.

RNA synthesis protocol:

Before setup of reaction, it is recommended to clean working area, wearing gloves, use of nuclease free tubes and water. All the components of kit should be seal tightly during incubation steps.

A. Synthesis of IVT RNA

1. Set up the transcription reaction at room temperature by adding the reagents indicated below in the table.

Components	Amount
RNase free water	Variable
Template DNA	0.5-1 µg
10X transcription Buffer	2 µl
100mM ATP	1.5 µl
100mM CTP	1.5 µl
100mM GTP	1.5 µl
100mM UTP	1.5 µl
100mm DTT	2 µl
RNase inhibitor	0.5 µl
T7 RNA polymerase	2 µl
Total reaction volume	**20 µl**

Important note: *Transcription buffer may result in formation of white precipitate. To dissolve heat at 37°C for 5min and mix thoroughly.*

2. Incubate at 37°C for 2hour (If a reaction is set in pcr tube then it can be incubated in pcr machine, if using 1.5ml tube for reaction then it can be incubated in incubator).

B. DNase I treatment of IVT reaction: DNase I treatment is use to remove DNA template from the IVT reaction.

1. Add 2µl of *10X DNAse I Reaction* buffer and 1µl of DNase I into 20µl of reaction mix from step A .
2. Mix gently
3. Incubate at 37°C for 15 minutes.
4. Proceed for RNA purification.

Purification of Synthetic RNA:

A. Ammonium precipitation method

1. Add one volume of 5 M ammonium acetate (21 µl for the standard reaction), mix well.
2. Incubate for 15 minutes on ice.
3. Pellet the RNA by centrifugation at >10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant with a pipette and gently rinse the pellet with 70% ethanol.
5. Remove the 70% ethanol with a pipette without disturbing the RNA pellet.
6. Allow pellet to dry, then resuspend in RNase-Free Water, TE or another suitable buffer.
7. Allow the pellet to dry, then resuspend in 50-75 µl of RNase-Free Water for quantitation.
8. Quantitate the RNA by spectrophotometry or fluorimetry. If desired, adjust the concentration of the RNA with RNase-Free Water. The RNA can now be frozen and stored at –20°C or –70C.

B. Ethanol precipitation method

1. Add 2.5–3.0 volumes of ice-cold ethanol (or 1 volume of isopropanol) and mix the solution well. Store the ethanolic solution for 1 h to overnight at −20℃ to allow the RNA to precipitate.
2. Recover the RNA by centrifugation at 12,000g–14,000g for 10 min at 4°C.
3. Decant the supernatant, and carefully remove remaining traces of the supernatant with an automatic micropipette or with a disposable pipette tip attached to a vacuum line. Take care not to disturb the pellet of nucleic acid (which may be invisible). Use the pipette tip to remove any drops of fluid that adhere to the walls of the tube.
4. Wash the pellet with 0.5 mL of ice-cold 70% ethanol and centrifuge at maximum speed for 10 min at 4℃ in a microcentrifuge.
5. Repeat Step 3.
6. Store the open tube on the bench at room temperature until the last traces of fluid have evaporated.
7. Dissolve the RNA pellet (which is often invisible) in the desired volume of RNase-free buffer (usually TE; pH between 6 and 7). Rinse the walls of the tube well with the buffer.

Evaluation of RNA product:

A. Quantification by Nanodrop

A Nanodrop Spectrophotometer can read RNA concentrations from 10 ng/µl to 3000 ng/µl directly. If quantifying using UV-visible spectrophotometer then it is necessary to dilute RNA sample (1:2000).

B. Analysis by Gel electrophoresis

Denaturing agarose gel: To make 100 mL 1% denaturing agarose gel, add 1 gram agarose powder to 72 mL nuclease-free water. Melt the agarose, add 10 ml 10X MOPS buffer. Then in a fume hood, add 18 ml fresh formaldehyde (37%), mix well. Pour the gel. 10X MOPS gel running buffer: 0.4 M MOPS (pH 7.0), 0.1 M Sodium Acetate, 10 mM EDTA.

Gel image of IVT RNA

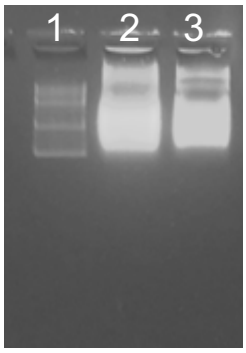


fig1: well no. 2&3 represent RNA formation from two different genes

Troubleshoot:

- **RNA Transcript of Larger Size than Expected**
  If the RNA transcript appears larger than expected on a denaturing gel, template plasmid DNA may be incompletely digested. Even small amounts of undigested circular DNA can produce large amounts of long transcripts. Check template for complete digestion, if undigested plasmid is confirmed, repeat restriction enzyme digestion. Larger size bands may also be observed when the RNA transcript is not completely denatured due to the presence of strong secondary structures.
- **RNA Transcript of Smaller Size than Expected**
  If denaturing gel analysis shows the presence of smaller bands than the expected size, it is most likely due to premature termination by the polymerase. Some sequences which resemble T7 RNA Polymerase termination signals will cause premature termination. Incubating the transcription reaction at lower temperatures, for example at 30°C, may increase the proportion of full-length transcript, however the yield will be decreased. For GC rich templates, or templates with secondary structures, incubation at 42°C may improve yield of full-length transcript.
- **RNA Transcript Smearing on Denaturing Gel**
  If the RNA appears degraded on denaturing agarose or polyacrylamide gel, DNA template is contaminated with RNase.
  DNA templates contaminated with RNase can affect the length and yield of RNA synthesized (a smear below the expected transcript length). If the plasmid DNA template is contaminated with RNase, perform phenol/chloroform extraction, then ethanol precipitate and dissolve the DNA in nuclease-free water
- **Low Yield of Full-length RNA**
  If the transcription reaction with your template generates full-length RNA, but the yield is significantly lower than expected, it is possible that contaminants in the DNA template are inhibiting the RNA polymerase, or the DNA concentration may be incorrect. Alternatively, additional purification of DNA template may be required. Phenol-chloroform extraction is recommended.
- **Low Yield of Short Transcript**
  High yields of short transcripts (< 0.3 kb) are achieved by extending incubation time and increasing the amount of template. Incubation of reactions up to 16 hours (overnight) or using up to 2µg of template will help to achieve maximum yield.