Evaluation of IVT Reaction Products

Materials Required but not Supplied

HiScribe® T7 ARCA mRNA Kit
- Nuclease-free Water (NEB #B1500)

HiScribe® T7 ARCA mRNA Kit (with tailing)
- Nuclease-free Water (NEB #B1500)

HiScribe® T7 mRNA Kit with CleanCap® Reagent AG
- Nuclease-free Water (NEB #B1500)

T7 RNA Polymerase
- RNase Inhibitor, Murine (NEB #M0314) (optional)
- Ribonucleotide Solution Mix (NEB #N0466)
- Ribonucleotide Solution Set (NEB #N0450)
- Nuclease-free Water (NEB #B1500)
- DTT

T7 RNA Polymerase (High Concentration)
- RNase Inhibitor, Murine (NEB #M0314) (optional)
- Ribonucleotide Solution Mix (NEB #N0466)
- Ribonucleotide Solution Set (NEB #N0450)
- Nuclease-free Water (NEB #B1500)
- DTT

Hi-T7® RNA Polymerase
- RNase Inhibitor, Murine (NEB #M0314) (optional)
- Ribonucleotide Solution Mix (NEB #N0466)
- Nuclease-free Water (NEB #B1500)

T3 RNA Polymerase
- RNase Inhibitor, Murine (NEB #M0314) (optional)
- Ribonucleotide Solution Mix (NEB #N0466)
- Fresh DTT (optional)
- Nuclease-free Water (NEB #B1500)

SP6 RNA Polymerase
- RNase Inhibitor, Murine (NEB #M0314) (optional)
- Ribonucleotide Solution Mix (NEB #N0466)
- Fresh DTT (optional)
- Nuclease-free Water (NEB #B1500)
- Ribonucleotide Solution Set (NEB #N0450)

Overview

In vitro transcription (IVT) reaction products generated by the HiScribe Kits or individual standalone RNA polymerases and NTPs can be evaluated for RNA concentration by absorbance and transcript product length by gel electrophoresis.

Quantification by UV Light Absorbance

RNA concentration can be determined by measuring the ultraviolet light absorbance at 260 nm. However, any unincorporated nucleotides and template DNA in the mixture will affect the reading, so must be removed before the RNA concentration can be quantified. Use recommended protocols for RNA purification.

A Nanodrop spectrophotometer can directly read RNA concentrations from 10 ng/µl to 3000 ng/µl; it may be necessary to dilute your RNA prior to measurement. For single-stranded RNA, 1 A_{260} is equivalent to an RNA concentration of 40 µg/ml. The RNA concentration can be calculated as follows: A_{260} x dilution factor x 40 = __ µg/ml RNA.

Analysis of Transcription Products by Gel Electrophoresis

To evaluate transcript length, integrity and quantity, an aliquot of the transcription reaction should be run on an appropriate denaturing agarose or polyacrylamide gel. Transcripts larger than 0.3 kb can be run on agarose gels, whereas denaturing polyacrylamide gels (5–15%) are necessary for smaller transcripts. The gels should be run under denaturing conditions to minimize formation of secondary structures by the transcript.

1. Preparation of denaturing gels and samples

1a. Denaturing agarose gel:

It is common practice to electrophorese RNA on a fully denaturing agarose gel, such as one containing formaldehyde. However, in many cases it is possible to run RNA on a native agarose gel and obtain suitable results. In fact, it has been demonstrated that treatment of RNA samples in a denaturing buffer (outlined below) maintains the RNA molecules in a denatured state for at least 3 hours during electrophoresis. The use of native agarose gels eliminates problems associated with toxic chemicals and the difficulties encountered when staining and blotting formaldehyde gels.

1. Add sample to an equal volume of RNA Loading Dye, (2X) (NEB #B0363) and mix well.
2. Heat at 70°C 10 minutes to denature the RNA. Cooling prior to loading is optional.
3. Load the samples.

1b. Denaturing PAGE/Urea gel:

We recommend using commercially available premade gels and standard TBE gel running buffer (10X TBE buffer: 0.9 M Tris Base, 0.9 M Boric Acid, 20 mM EDTA).

2. Gel electrophoresis

1. Mix 0.2–1 µg RNA sample with 5–10 µl of RNA Loading Dye, 2X (NEB #B0363).
2. Denature the RNA sample and an aliquot of RNA marker by heating at 65–70°C for 5–10 minutes.
3. Pulse-spin prior to loading onto the gel.
4. Visualize RNA by staining the gel post-electrophoresis with SYBR® Gold (preferred) or ethidium bromide.

Related Resources

- Avoiding Ribonuclease Contamination
- A Practical Guide to Analyzing Nucleic Acid Concentration and Purity with Microvolume Spectrophotometers