Purification of IVT RNA

Materials Required but not Supplied

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

HiScribe® T7 ARCA mRNA Kit
- 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 general, RNA synthesized by *in vitro* transcription can be purified by LiCl precipitation, phenol-chloroform extraction followed by ethanol precipitation, or by using a spin column-based method. If absolute full-length RNA is required, we recommend gel purification. For capped RNA, non-radioactively labeled RNA, or high specific activity radiolabeled RNA probes, spin column chromatography is the preferred method.

LiCl Precipitation

LiCl precipitation can be used for quick recovery of the synthesized RNA and is an effective method for removing the majority of unincorporated NTPs and enzymes. However, RNAs shorter than 300 bases or at concentrations lower than 0.1 mg/ml do not precipitate well. In such cases, other purification methods may be used. A tube of LiCl Solution (7.5 M LiCl, 10 mM EDTA) is provided with NEB #E2050, #E2060, #E2065, #E2070, and #E2080.

Protocol

1. Adjust the reaction volume to 50 µl by adding nuclease-free water.
2. To the 50 µl IVT reaction product, add 25 µl LiCl solution and mix well.
3. Incubate at –20°C for 30 minutes.
4. Centrifuge at 4°C for 15 minutes at top speed to pellet the RNA.
5. Remove the supernatant carefully.
6. Rinse the pellet by adding 500 µl of cold 70% ethanol and centrifuge at 4°C for 10 minutes.
7. Remove the ethanol carefully. Spin the tube briefly to bring down any liquid on the wall.
8. Remove residual liquid carefully using a sharp tip (e.g., loading tip).
9. Air dry the pellet and resuspend the RNA in 50 µl of 0.1 mM EDTA or a suitable RNA storage solution.
10. Heat the RNA at 65°C for 5-10 minutes to completely dissolve the RNA. Mix well.
11. Store the RNA at –20°C or below.

Phenol-Chloroform Extraction and Ethanol Precipitation

For removal of proteins and most of the free nucleotides, phenol-chloroform extraction and ethanol precipitation of RNA transcripts is the preferred method.

Protocol

1. Adjust the reaction volume to 180 µl by adding nuclease-free water. Add 20 µl of 3 M sodium acetate (pH 5.2) or 20 µl of 5 M ammonium acetate and mix thoroughly.
2. Extract with an equal volume of 1:1 phenol:chloroform mixture, followed by two extractions with chloroform. Collect the aqueous phase and transfer it to a new tube.
3. Precipitate the RNA by adding 2 volumes of ethanol. Incubate at –20°C for at least 30 minutes and collect the pellet by centrifugation.

4. Remove the supernatant carefully.

5. Rinse the pellet by adding 500 µl of cold 70% ethanol and centrifuge at 4°C for 10 minutes.

6. Remove the ethanol carefully. Spin the tube briefly to bring down any liquid on the wall.

7. Remove residual liquid carefully using a sharp tip (e.g., loading tip).

8. Air dry the pellet and resuspend the mRNA in 50 µl of 0.1 mM EDTA or a suitable RNA storage solution.

9. Store the mRNA at –20°C or below.

Spin Column Chromatography

Spin columns will remove unincorporated nucleotides, proteins, and salts. We recommend using a Monarch Spin RNA Cleanup Kit (10 µg capacity NEB #T2030, 50 µg capacity #T2040, 500 µg capacity #T2050 or 3 mg high-capacity #T2060) with the Monarch® Spin RNA Cleanup Kit Protocol.

Adjust the volume of the reaction mixture to 100 µl by adding nuclease-free water to the IVT product and mix well. Purify the RNA by following the manufacturer's instructions. Be aware that some reactions could produce 180 µg of RNA, which may exceed individual column capacity and require additional columns.

Gel Purification

When high purity RNA transcript is desired (such as making RNA probe for RNase protection assays), we recommend gel purification of the transcription product. Gel purification of mRNA tailed by Poly(A) Polymerase is not recommended because the tailed mRNA is heterogeneous in length and will result in a smear on the gel. If the poly(A) tail is encoded in the DNA template, the RNA should resolve into a single band for gel purification.

The Monarch Spin RNA Cleanup Kits (NEB #T2030, #T2040, or #T2050) can be used for extraction of RNA from gels, although this is not their primary application, so recoveries may range from 40-70% (see protocol included in the product manuals).

Related Resources

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