Promega

Product Application

Clean-Up of *In Vitro* Transcription Reactions Using the ReliaPrep™ RNA Clean-Up and Concentration System

Clean-up in vitro transcription reaction rapidly and without phenol chloroform extraction

Kit: ReliaPrep™ RNA Clean-Up and Concentration System (Z1073)

Analyses: NanoDrop™, QuantiFluor RNA System, TapeStation

Sample Type(s): *In vitro* transcription reactions

Input: 20µL

Materials Required:

- Microcentrifuge
- Vortex
- 100% Isopropanol
- 95-100% Ethanol

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TB298, available at: www.promega.com/protocols

or by e-mailing Technical Services techserv@promega.com

Protocol:

1. Pipet 20µL of in *in vitro* transcription reactions into a 1.5mL microcentrifuge tube.
2. Add 12.5µL of Membrane Binding Solution and vortex for 5 seconds.
3. Add 37.5µL of 100% isopropanol.
4. Load RNA sample onto a ReliaPrep™ Minicolumn seated in a Collection Tube and centrifuge for 30 seconds.
5. Remove column, and discard the contents of the Collection Tube. Reseat the column into the same Collection Tube.
6. Add 200µL of Column Wash Solution (CWE) and centrifuge for 15 seconds. Remove column, and discard the contents of the Collection Tube. Reseat the column into the same Collection Tube.
7. Wash with 300µL of RNA Wash Solution (RWA) and centrifuge for 15 seconds. Repeat wash with 300µL of RNA Wash Solution (RWA) and centrifuge again.
8. Remove column and discard the contents of the collection tube. Reseat the column into the same collection tube, centrifuge for 1 minute to dry the column and then transfer the column to an Elution Tube.
9. Pipet 15µL of Nuclease-Free Water to the center of the column matrix and centrifuge for 30 seconds.
10. For maximum recovery, repeat elution with an additional 15µL of Nuclease-Free Water.

Results:

RNA was transcribed from the pGEM® Express Positive Control Template using the T7 RiboMax™ Express Large-Scale RNA Production System following the standard protocol (TB298). Reactions were purified using phenol chloroform extraction as recommended by the technical manual or the ReliaPrep™ RNA Clean-Up and Concentration System (TM541). One of the phenol chloroform extractions was not included in this data set due to phenol contamination.

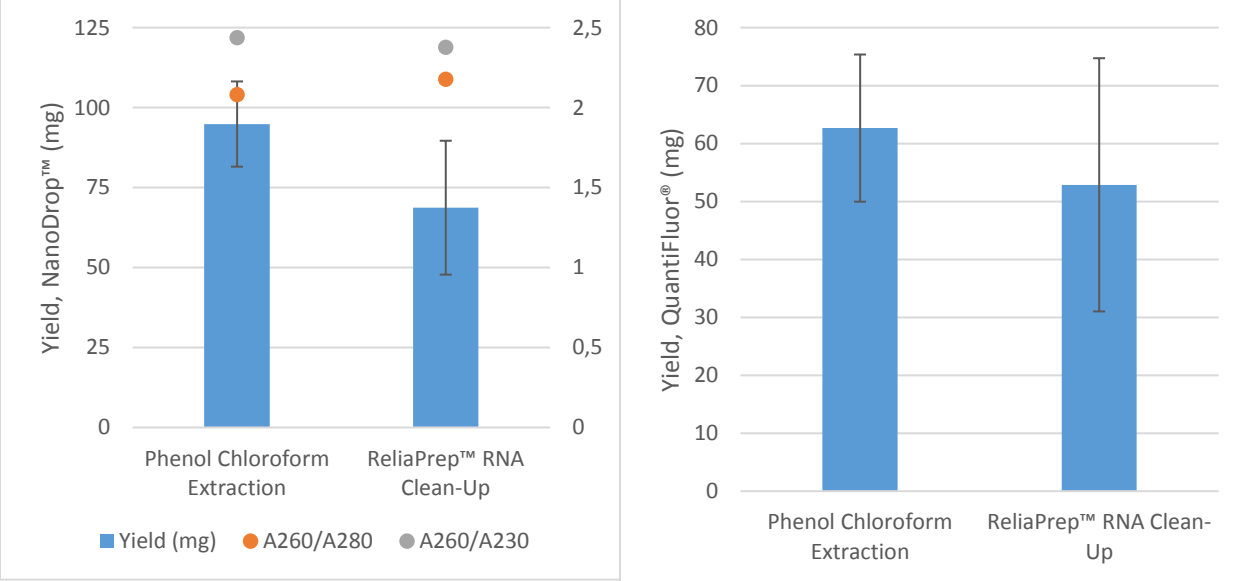

Figure 1. DNA yield measured by absorbance using NanoDrop™ (left) and QuantiFluor® RNA System (right) for *in vitro* transcription reactions purified using phenol chloroform extraction or the ReliaPrep™ Clean-Up and Concentration System. Mean ± Standard Deviation of n=3 for phenol chloroform extraction and n=4 for ReliaPrep™ RNA Clean-Up and Concentration System are shown.

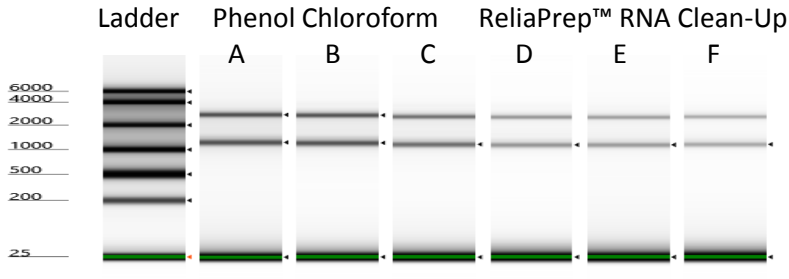

Figure 2. RNA integrity for *in vitro* transcription reactions purified using phenol chloroform extraction (A-C) or the ReliaPrep™ Clean-Up and Concentration System (D-F). RNA integrity was tested using RNA ScreenTape on an Agilent 4200 TapeStation according to the technical manual as shown above.