RNA Cleanup with the ProNex® Size-Selective Purification System

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Materials Required

- ProNex® Size-Selective Purification System (Cat.# NG2001)
- 95–100% ethanol
- magnetic stand
- single or multichannel 20–200µl pipette and tips
- single or multichannel 100–1000µl pipette and tips (optional)
- buffer trough (if using a multichannel pipette)
- 96-well plate capable of holding up to 5X the starting sample volume if working in a multichannel format

A magnetic bead-based technique can provide a simple, fast, effective and automatable method for RNA cleanup.

Introduction

Producing pure RNA is critical for many applications, including RT-qPCR, microarrays and the early stages of library preparation for RNAseq. Amplification inhibitors carried over from extraction and impurities from enzymatic reactions such as DNase digestions should be removed prior to these sensitive assays. A magnetic bead-based technique can provide a simple, fast, effective and automatable method for RNA cleanup. This application note describes a protocol for using the ProNex® Size-Selective Purification System to clean up RNA preparations.

Note: While bulk purification of RNA and ssDNA is possible with the ProNex® Size-Selective Purification System, size-selective purification of RNA and ssDNA is not possible with this system.

Protocol

This protocol can be used to purify RNA of ~100nt or larger from contaminants (e.g., buffers, proteins, salts, etc.) and low-molecular-weight nucleic acid fragments (e.g., adapters, oligonucleotides and nucleotides).

Note: This protocol is based on a 50µl starting sample volume.

Before you begin: Reconstitute the Wash Buffer by adding 95–100% ethanol to the wash buffer provided in the kit.

1. Allow the ProNex® Size-Selective Chemistry to equilibrate to room temperature for 30 minutes to 1 hour.
2. Pipette sample into a tube or well capable of holding the final reaction volume.
3. Ensure that the ProNex® Size-Selective Chemistry bottle cap is tightened securely. Resuspend the resin by vigorous vortexing for 10 seconds or longer.
4. Mix ProNex® Size-Selective Chemistry into the RNA sample at a 3:1 vol:vol ratio of ProNex® Chemistry to sample (e.g., add 150µl of ProNex® Size-Selective Chemistry to 50µl of RNA sample) by pipetting 10 times.
5. Incubate the sample at room temperature for 10 minutes.
6. Place the sample on a magnetic stand for 2 minutes.
7. Carefully remove and discard the supernatant.

8. Leaving the sample on the magnetic stand, add 200µl of reconstituted Wash Buffer to the sample and incubate for 30–60 seconds. Remove and discard the Wash Buffer. For larger samples, increase the volume of Wash Buffer proportionally to the total volume of sample and ProNex® Size-Selective Chemistry.
9. Repeat Step 8 for a total of 2 washes.
10. Allow the sample to air-dry for 5 minutes.
    Notes:
    1. If working with a multichannel pipette and buffer trough, return the unused Wash Buffer to the Wash Buffer bottle at this point. Tighten the bottle cap to prevent ethanol evaporation.
    2. The resin may be allowed to air-dry for longer than 5 minutes. Depending on the sensitivity of downstream applications to ethanol, drying times up to 1 hour can be used.
11. Remove the sample from the magnetic stand.
12. Add 50µl of Elution Buffer, and resuspend the resin by pipetting or shaking on a plate mixer. Incubate the samples at room temperature for 5 minutes to elute the RNA.
    Note: More or less Elution Buffer may be used. Higher elution volumes do not result in significant yield increases; however, elution volumes <25% of the starting sample volume can be difficult to work with and may result in some yield loss due to the resin void volume.
13. Return the sample to the magnetic stand for 1 minute, and then carefully transfer the eluted RNA to a clean tube or well.
14. Return the ProNex® Size-Selective Chemistry bottle to storage at 2–10°C.

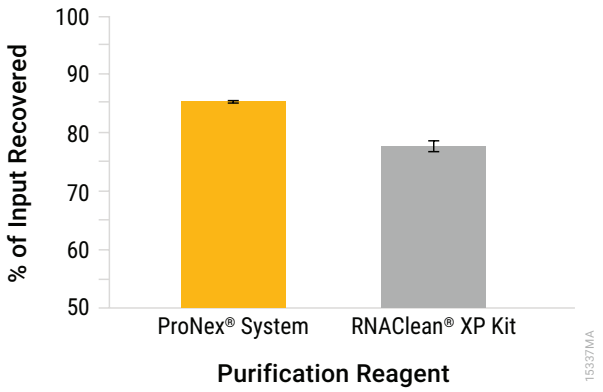

Figure 1. Side-by-side comparison of two magnetic bead-based RNA purification chemistries. Fifty microliters of pre-purified RNA was purified with the ProNex® Size-Selective Purification System and the Agencourt® RNAClean® XP Kit using their respective protocols.