

High-throughput, automated purification of DNA-free RNA directly from samples in TRIzol®, TRI Reagent®, or similar without phase separation.

Introduction

The Direct-zol™-96 MagBead RNA facilitates purification of high quality (DNA-free) RNA directly from samples stored in TRIzol®, TRI Reagent® or similar reagents. While exceptional in providing RNA stabilization and inactivating infectious agents, these reagents are complicated by phase separation, precipitation, and potential phenol carryover. The innovative, Direct-zol™ procedure from Zymo Research bypasses phase separation/precipitation requirements and eliminates phenol carryover. The Direct-zol™-96 MagBead RNA meets the demands of scientists requiring high-quality RNA for sensitive analytical methods like miRNA profiling, RNA-seq, and viral detection.

Automation Equipment

- Tecan Freedom EVO®
- Freedom EVOware®
- 8 channel Liquid Handling Arm (LiHa),
- configured for Disposable Tips (DiTis)
- Robotic Manipulation Arm (RoMA)
- Te-Shake™ Shaker
- 96-well Magnetic Stand

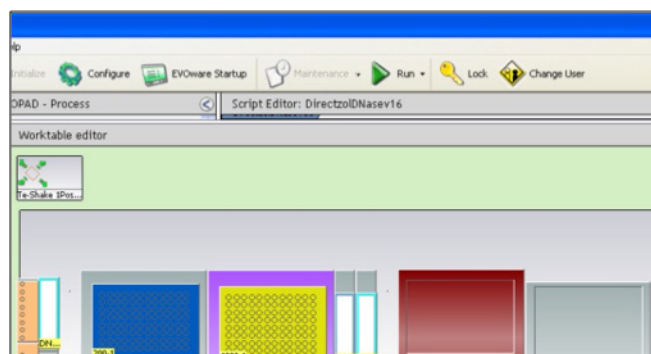
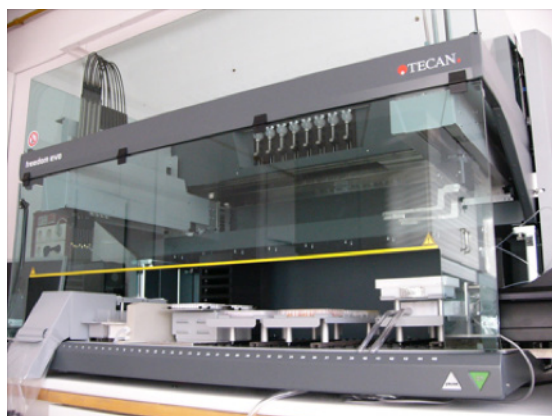


Figure 1. Example Deck Layout

Overview of Procedure

Simply add Direct-zol™ Binding Buffer and MagBinding Beads to a sample in TRI Reagent® to bind RNA to the magnetic beads, then wash, DNase treat, and elute the RNA. The extraction method inactivates viruses and other potentially infectious agents. No phase separation, precipitation, or post-purification steps are necessary. DNA-free total RNA, including small and non-coding RNAs (17-200 nt), can be effectively isolated from a variety of sample sources including cells, tissues, serum, plasma, blood, biological liquids, etc.

Results

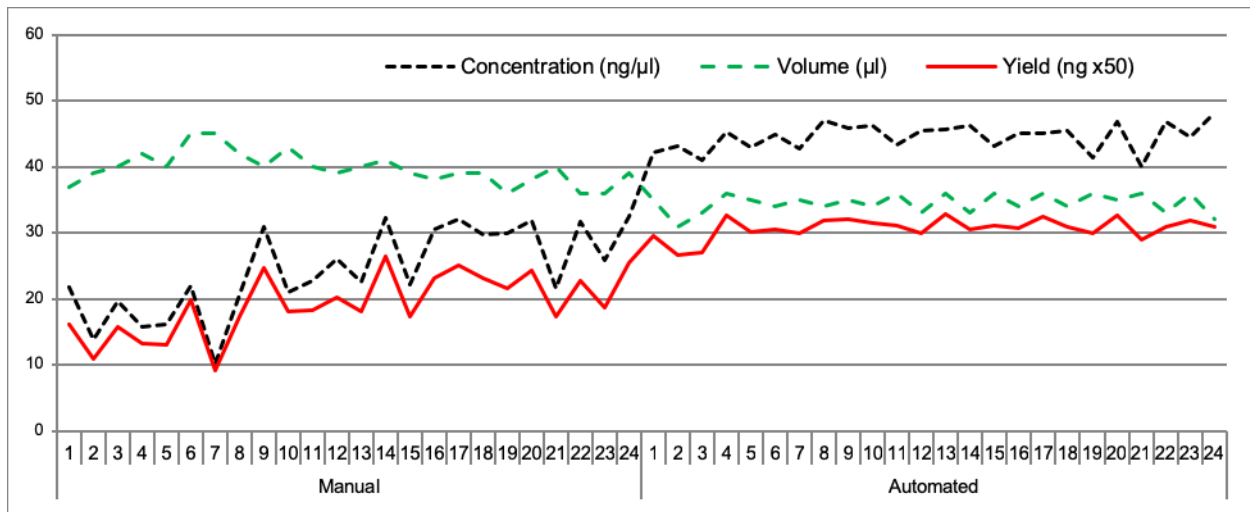


Figure 2. Automation results in highly reproducible RNA yields. Total RNA was purified from human epithelial cells (5.0×10^5 /well) using the Direct-zol™-96 MagBead RNA on a Freedom EVO®. Data show the comparison of concentration, recovery volume, and total yield of manual vs. automated processing for replicate samples across a 96-well plate.

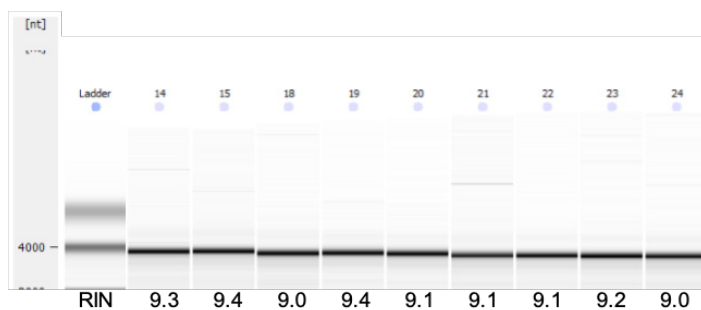


Figure 3. RNA purified is high quality. Total RNA (including small RNAs) was purified from human epithelial cells (5.0×10^5 /well) using the Direct-zol™-96 MagBead RNA and analyzed by the Agilent Bioanalyzer 2100 (RNA 6000 Nano Chip).

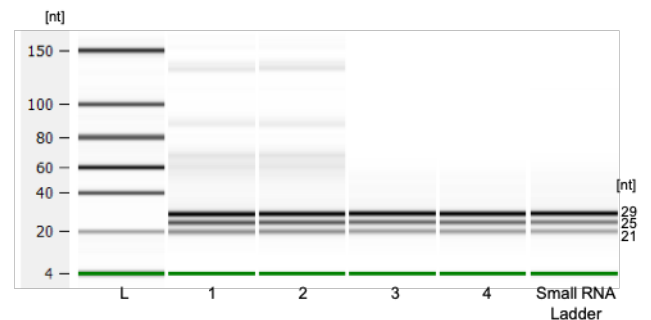


Figure 4. Small RNAs are efficiently recovered as analyzed by the Agilent Bioanalyzer 2100 (Small RNA Chip). Total RNA including small RNAs (1, 2), Small RNAs only (3, 4).

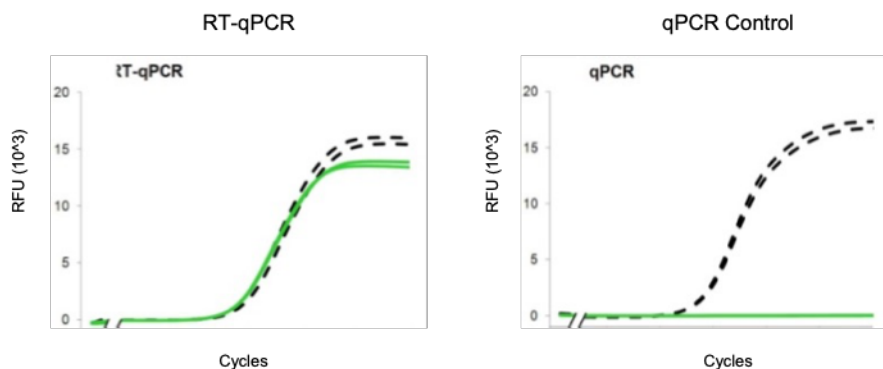


Figure 5. DNA-free RNA for reliable RT-PCR. RNA was isolated from human epithelial cells with the Direct-zol™-96 MagBead RNA (green) and detected by quantitative RT-PCR and PCR with human beta-actin primers. Non-DNase treated samples (black) are included for comparison.

Conclusions

The Direct-zol™-96 MagBead RNA is a high-throughput, automated RNA isolation method that exhibiting excellent reproducibility and consistency in volume and concentration. Automated processing yields greater consistency in total RNA recovery including small and non-coding RNAs (17-200 nt) compared to manual processing. This innovative method is efficient for providing high-quality DNA-free RNA from samples in TRI Reagent® or similar. RNA is suitable for subsequent RNA-based methods including RT-PCR, transcription profiling, hybridization, etc.

Specifications

- Sample Sources – Any sample stored and preserved in TRIzol®, TRI Reagent®, or similar: animal cells, tissue and biological liquids (e.g. blood, plasma, serum). Also, compatible with in vitro processed RNA (e.g. transcription products, DNase-treated or labeled RNA) and samples in DNA/RNA Shield™.
- Purity – High-quality RNA is ready for Next-Gen sequencing, RT-PCR, hybridization, etc. Complete removal of DNA is performed with DNase I digestion.
- Binding Capacity – 10 µg RNA per 20 µl magnetic beads.
- Size – Total RNA including small/microRNAs (>17 nt).
- Elution Volume – ≥50 µl DNase/RNase-Free Water.
- Sample Inactivation – TRI Reagent® inhibits RNase activity and inactivates viruses and other infectious agents.

Product	Cat. No.	Kit Size
Direct-zol-96 RNA MagBead (TRI Reagent not included)	R2100 R2102	96 preps 4 x 96 preps
Direct-zol-96 RNA MagBead (supplied with TRI Reagent)	R2101 R2103	96 preps 4 x 96 preps



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