

## 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

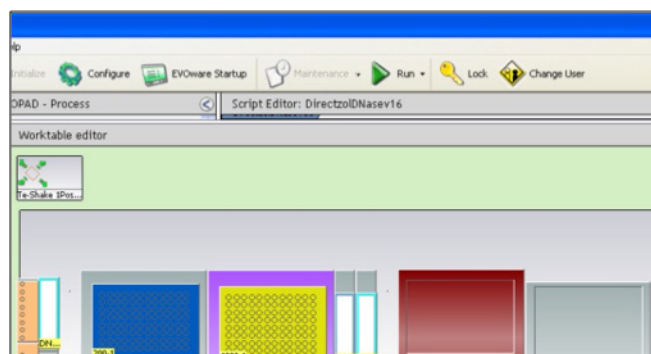
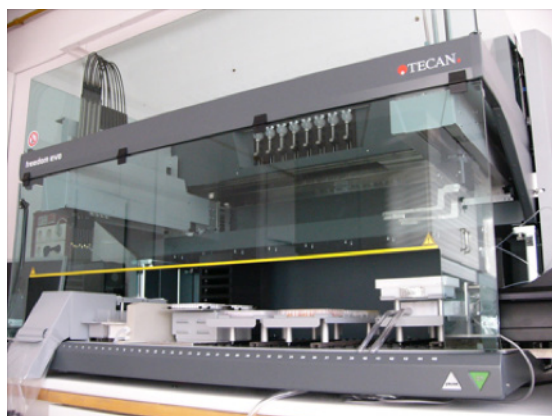
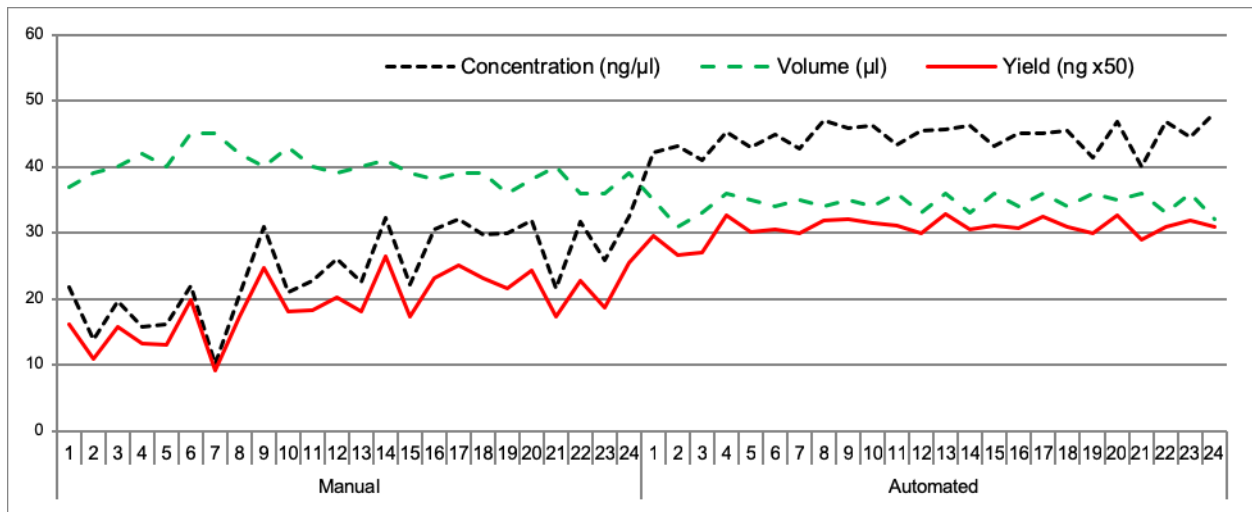


Figure 1. Example Deck Layout

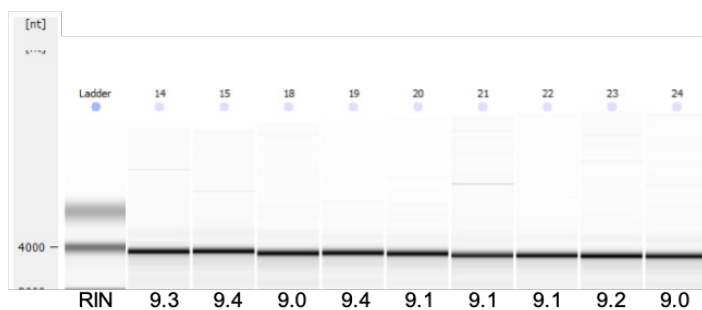
## Overview of Procedure

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.

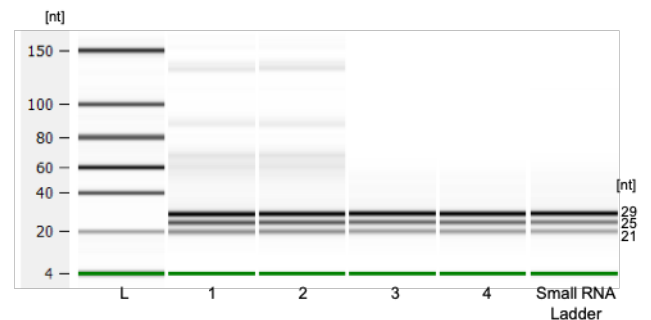
## Results



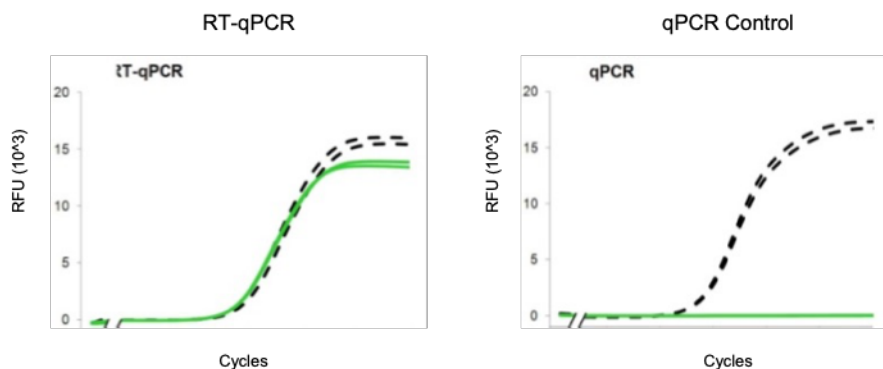
**Figure 2. Automation results in highly reproducible RNA yields.** Total RNA was purified from human epithelial cells ( $5.0 \times 10^5$ /well) using the Direct-zol™-96 MagBead RNA on a Freedom EVO®. Data show the comparison of concentration, recovery volume, and total yield of manual vs. automated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