Automation of the Direct-zol™-96 MagBead RNA

High-throughput, magnetic bead-based purification of DNA-free RNA *directly* from samples in TRIzol® without phase separation on the Microlab® STAR™

Introduction

The TRIzol method for RNA extraction has been the gold standard. The powerful protein denaturant effectively stabilizes RNA and inactivates RNases and infectious agents. However, the need for phase separation, precipitation, and potential phenol carryover can further complicate workflows. The magnetic bead-based Direct-zol procedure on the Hamilton Microlab STAR platform bypasses phase separation/precipitation and enables high-throughput automated magnetic bead-based purification of high-quality total RNA directly from samples treated in TRIzol or other acid-guanidinium-phenol based reagents. Direct-zol effectively isolates total RNA from a variety of sample sources including cells, tissue, serum, plasma, blood, and biological liquids for downstream applications, like miRNA profiling, RNA-seq, and viral detection.

Materials and Methods

Forty-eight samples of HeLa cells ($5x10^5$/sample) were treated with TRIzol following published protocol and used as input samples. Total RNA was extracted from the HeLa samples with Zymo Direct-zol-96 MagBead RNA kit (catalog #R2100) using the extraction workflow (Figure 1, page 2). Twenty-four of the HeLa samples were processed manually and the other 24 were processed using a Hamilton Microlab STAR.

The Microlab STAR used was configured with 8 x 5 mL Independent Pipetting Channels, Autoload, CO-RE® 96 Multi-Probe Head (MPH), CO-RE Grippers, Hamilton Heater Shaker (HHS), Zymo magnetic rack, as well as required tips, and reagent carriers.

The RNA concentration was analyzed using Thermo Scientific NanoDrop® 2000 UV-Vis Spectrophotometer. RNA purity was analyzed using Agilent Bioanalyzer™ 2100 (RNA 6000 Nano Chip). Efficient recovery of small RNA was analyzed using Agilent Bioanalyzer 2100 (small RNA Chip).

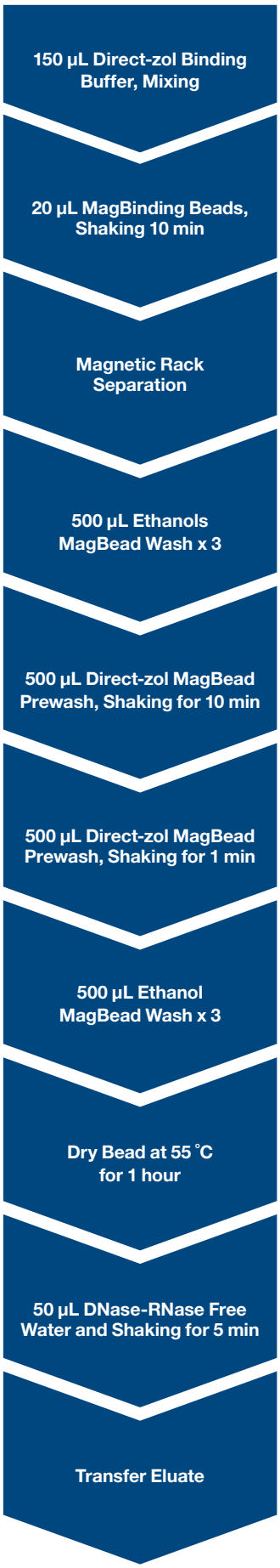

Figure 1: Zymo Direct-zol-96 MagBead RNA extraction workflow.

Results and Discussion

Consistent Yields and High Quality

RNA concentration, recovered volume, and total RNA yields from replicate HeLa cells ($5x10^5$/well) were compared between 24 manually processed samples and 24 samples processed on the Hamilton Microlab STAR. The results indicate that automation is comparable with the manual process (Figure 2).

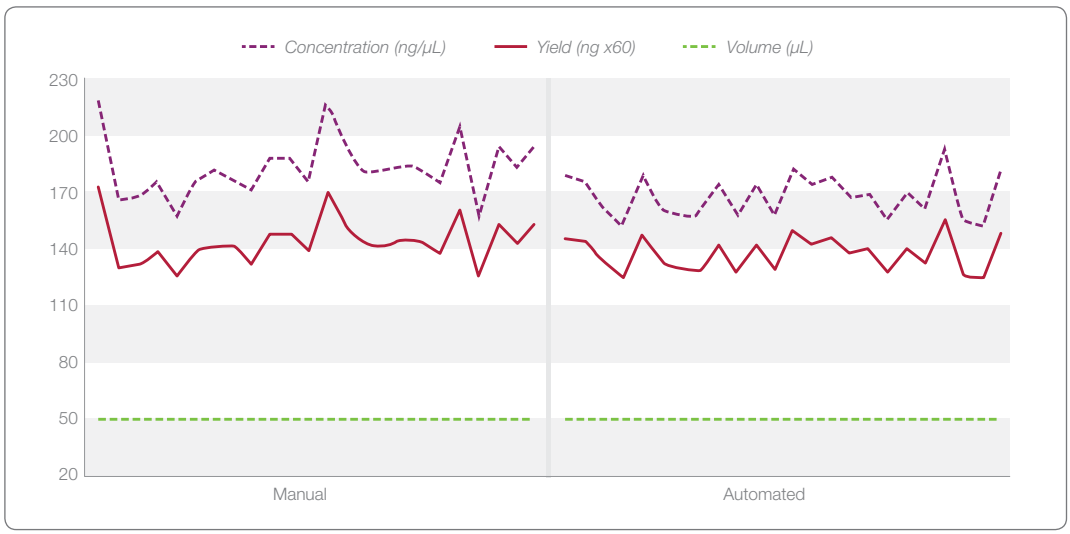

Figure 2: Comparison between manual and automated (Microlab STAR) sample processing.

Total RNA Purity

Randomly selected samples from the 24 processed on the Hamilton Microlab STAR were applied to a BioAnalyzer RNA Nano 6000 Nano Chip to determine RNA purity. The results show homogenous quality of purified total RNA in all selected samples (Figure 3).

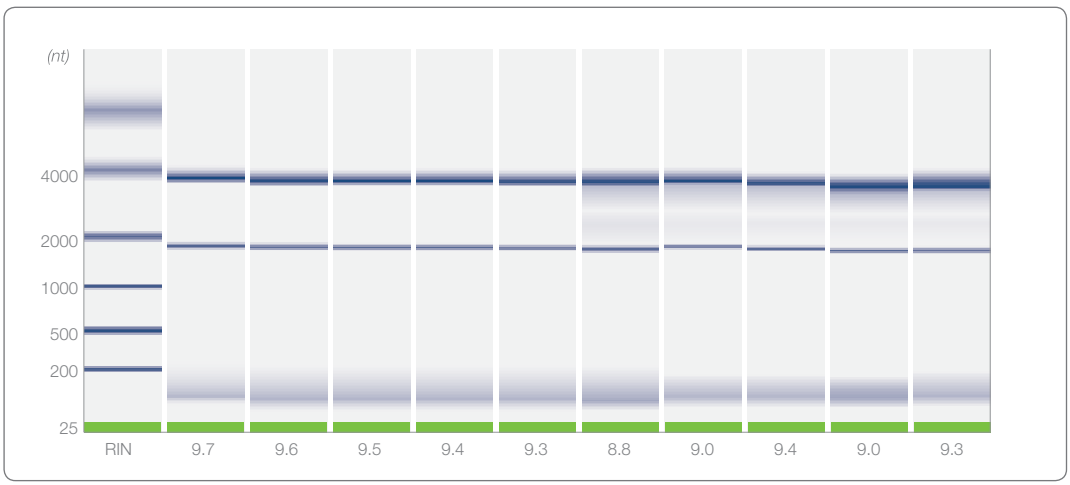

Figure 3: Purified total RNA (including small RNAs) is analyzed by the Agilent Bioanalyzer 2100.

Efficient Recovery of Small RNA

Recovery of small RNAs, including non-coding and miRNA (17 – 200 nt) from total RNA extracted samples, is shown to be non-biased (Figure 4).

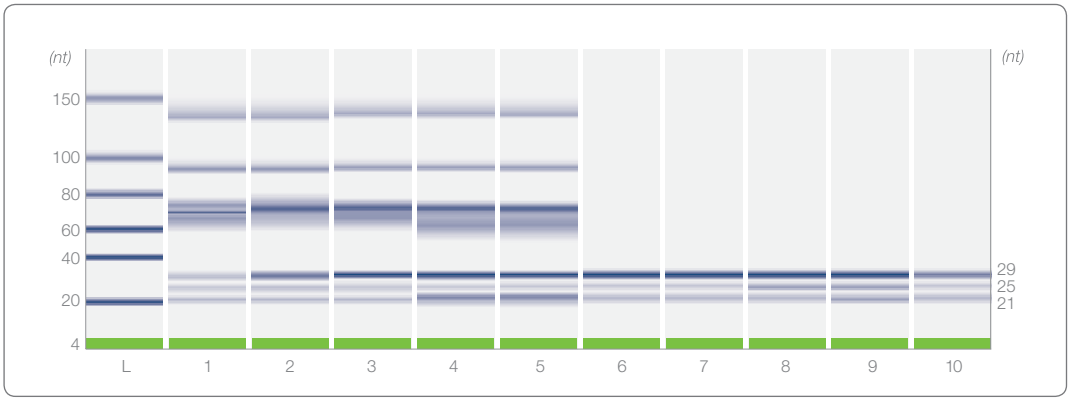

Figure 4: Purified small RNAs are recovered as analyzed by the Agilent Bioanalyzer 2100 (small RNA Chip). Total RNA including small RNAs (1 – 5), small RNAs only (6 – 10).

Conclusions

Samples processed using the Direct-zol-96 MagBead RNA procedures with the Microlab STAR perform comparably with manual pipetting techniques and methods. This is shown by the successful recovery and excellent reproducibility and consistency in volume and concentration. This innovative method yields high-quality DNA-free RNA (including small RNAs) from samples in TRIzol or similar reagent, providing an efficient solution for reliable high-throughput hands-free RNA purification.

Zymo Products

Product Description	Catalog No.	Kit Size
Direct-zol-96 RNA MagPrep (TRI Reagent not included)	R2100	2 x 96 preps.
	R2102	4 x 96 preps.
	R2104	8 x 96 preps.
Direct-zol-96 RNA MagPrep (supplied with TRI Reagent)	R2101	2 x 96 preps.
	R2103	4 x 96 preps.
	R2105	8 x 96 preps.

Lit. No. L50165 v1.0 — 04/2017

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