ZYMO RESEARCH

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Automated TRIzol® RNA Extraction without Phase Separation on the KingFisher Flex

High-throughput, magnetic bead-based RNA extraction from any TRIzol® sample without phase separation using the Direct-zol™-96 MagBead RNA.

Introduction

The Direct-zol™-96 MagBead RNA kit harnesses the familiar and cited technology of TRIzol® and acid guanidinium-phenol chemistry, but without all of its negative aspects such as chloroform addition, precipitation, phenol carryover, time consumption, etc. Using the Direct-zol™ method, RNA can be bound directly to magnetic beads without the need for the phase separation or centrifugation steps found in the TRIzol® method. High quality total RNA can be extracted from a variety of sample types including cells, tissue, serum, plasma, blood, and biological fluids and is usable for all downstream applications including miRNA profiling, RNA-seq, and viral detection.

The highlights of the Direct-zol™ chemistry lends itself to simple integration and automation on the KingFisher™ Flex (Thermo Fisher Scientific). The instrument can process up to 96 samples per run using magnetic rods that transfer beads from plate to plate holding various bind, wash, and elution buffers. Combined with the intuitive and user friendly BindIt™ software, the Kingfisher Flex and its open flexibility allows for easy optimization and customization of the Direct-zol™ workflow for maximum reproducibility and consistent results.

Materials and Methods

Equal 150 µL aliquots of $2.5x10^5$ HeLa cells were lysed in TRIzol® and were used as starting sample inputs. Half the samples were processed manually and the other half were processed using the KingFisher™ Flex (n=12).

Total RNA was extracted using the Direct-zol™-96 MagBead RNA kit (Cat. #R2100) following the published protocol. In summary, Direct-zol™ Binding Buffer and MagBinding Beads were added for binding, then a series of washes were performed to remove contaminants. DNase treatment was included in the middle of the procedure, then Direct-zol™ MagBead PreWash was used to re-bind RNA back onto the beads. Magnetic beads were dried at room temperature for 10 minutes and were eluted in 50 µL. The entire processing time on the KingFisher Flex™ is approximately 60 minutes.

Overall yield (µg) and sample purity (A260/280, A260/230) were determined by Thermo Scientific NanoDrop™ 2000 UV-Vis Spectrophotometer. RNA quality and integrity were analyzed using Agilent 2200 TapeStation® (High Sensitivity RNA ScreenTape).

Results and Discussion

RNA yield between the manual and automated workflows were very similar and in line with the typical expected yield of ~4 µg for $2.5x10^5$ HeLa cells (Figure 1). Both workflows resulted in A260/280 scores >2.0, but the KingFisher™ workflow slightly outperform the manual workflow resulting in cleaner elutions overall (Figure 1). The TapeStation® RNA profiles between the manual and automated processes were identical and both processes yielded high-quality total RNA with scores RIN > 9.0 (Figure 2).

Conclusions

Automation of the Direct-zol™-96 MagBead RNA kit with the KingFisher Flex yielded similar results and RNA quality to manual processing. Direct-zol™ is the optimal choice for automated TRIzol® based RNA extraction since onerous steps such as phase separation and lengthy centrifugation are completely bypassed; allowing for a streamlined solution that is reproducible and yields consistent results.

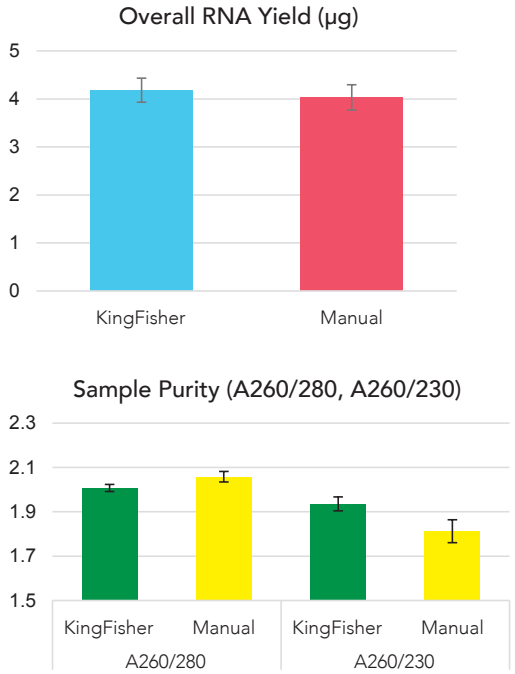

Figure 1. Yield and Purity between manual and automated (KingFisher) processing

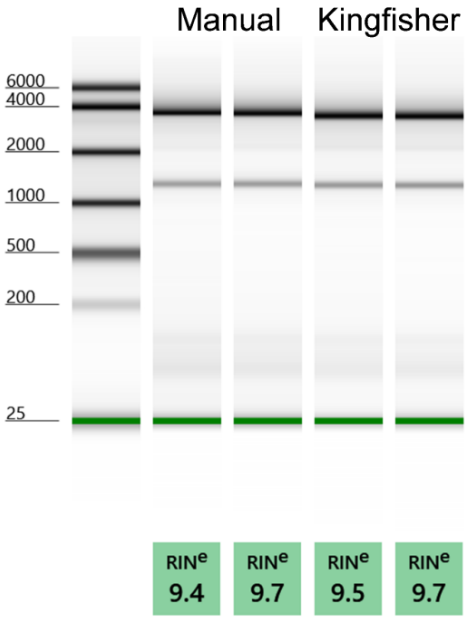

Figure 2. Extracted RNA is intact with high RIN scores. Quality assessed by Agilent 2200 TapeStation

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