Automated RNA extraction yields reproducible, high-throughput, quality results in samples stored in TRIzol®

ABSTRACT

Obtaining high quality, pure RNA is important for sensitive analyses like gene expression profiling in healthy and diseased samples. Inconsistencies in sample handling can lead to inaccurate results. In this study, the Opentrons OT-2 Nucleic Acid Extraction Workstation was used with the Zymo Research Direct-Zol™ 96 MagBead RNA kit to automate RNA extraction and purification. RNA quality, yield, and integrity were determined for 12 HeLa samples stored in TRIzol® and an assessment to detect cross-contamination between plate wells was also conducted. All RNA samples were of high concentration, purity, and integrity, and no cross-contamination was detected demonstrating successful extraction and purification of RNA using OT-2 automation of the Direct-Zol kit.

INTRODUCTION

Obtaining high quality, pure RNA in sufficient quantities will contribute to reproducible and reliable results. RNA must be purified to remove contaminants that can cause RNA degradation[1] or introduce bias or error due to the presence of DNA or PCR inhibitors.[2,3] TRIzol®/TRI Reagent® is commonly used for RNA stabilization and inactivation of infectious agents and RNase, but requires a tedious manual process using chloroform for phase-separation. Manual sample manipulation also introduces opportunities for contamination that can degrade RNA and increases variability due to intra- and inter-individual imprecision. The Zymo Research Direct-Zol 96 MagBead RNA kit eliminates the phase-separation step, and the magnetic beads allow for extraction to be automated with the Opentrons OT-2 Nucleic Acid Extraction Workstation.

The chemistry of the Direct-Zol kit enables this product to be easily integrated and automated on the Opentrons OT-2, allowing for high-throughput processing of up to 96 samples per run. In this study, the performance of the OT-2 Nucleic Acid Extraction Workstation in combination with the Direct-Zol 96 MagBead RNA kit was evaluated by extracting RNA from 12 individual HeLa cell samples. The extracted RNA was characterized for quality, yield, and integrity and no cross-contamination was detected between plate wells. This automation approach provided high-quality RNA while also significantly reducing the time and labor required for manual processing. Additionally, it can be added that the Direct-Zol kit's unique chemistry allows for efficient and effective purification of RNA from a wide range of sample types, eliminating the need for labor-intensive steps such as phase separation or RNA precipitation, and avoiding the use of harsh chemicals such as chloroform.

MATERIALS

Reagents	Automation Equipment
Direct-Zol 96 MagBead RNA Kit (Cat. #R2100)	OT-2
Ethanol (95-100%)	Magnetic Module GEN1
	8-Channel GEN2 P300 Pipette
	Opentrons 200 µL Filter Tips
	Skirted PCR Plate
	NEST® 2 mL 96-Well Deep Well Plate, V Bottom

METHODS

All RNA extraction steps were conducted on the OT-2 Nucleic Acid Extraction Workstation. First, 2.5x10^5 HeLa cells were lysed in 150µL of TRIzol®. The lysate was then mixed with ethanol and MagBinding Beads to bind the RNA. To remove contaminants, the RNA-bound beads were washed with MagBead DNA/RNA Wash 1, MagBead DNA/RNA Wash 2, and ethanol. Samples were treated with DNase to remove any residual DNA contamination. Next, Direct-Zol MagBead PreWash was added to re-bind RNA to the beads. A final ethanol wash was performed to remove any DNase. The MagBinding Beads were then dried at room temperature for 10 minutes and the RNA was eluted in 50µL of Elution Buffer. RNA yield and purity were determined by Thermo Scientific NanoDrop 2000 UV-Vis Spectrophotometer. RNA quality and integrity were analyzed using Agilent 2200 TapeStation®.

To determine if automation introduced cross-contamination, extracted RNA (Positive Control) and DNase/RNase Free-Water (Negative Control) were added to a 96-well plate in an alternating, checkerboard pattern. The plate was processed through the same Direct-Zol 96 MagBead RNA protocol using the Opentrons OT-2. The resulting RNA samples and Negative Controls were quantified by Qubit RNA High Sensitivity on the Qubit 3.0 Fluorometer.

RESULTS

Automated extraction resulted in high RNA concentration, quality, and integrity across all samples

The RNA yield across all samples ranged between 25.9 to 33.2ng/µL with an overall average of 28.5ng/µL **(Figure 1)**.

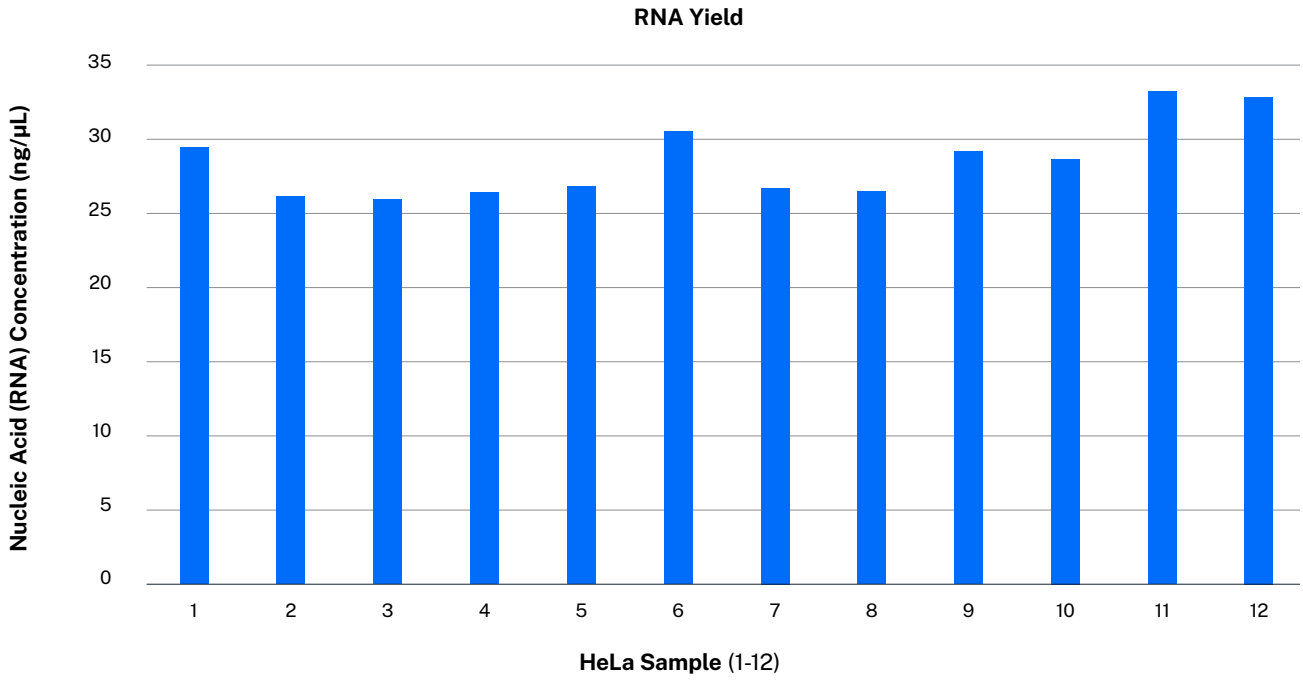
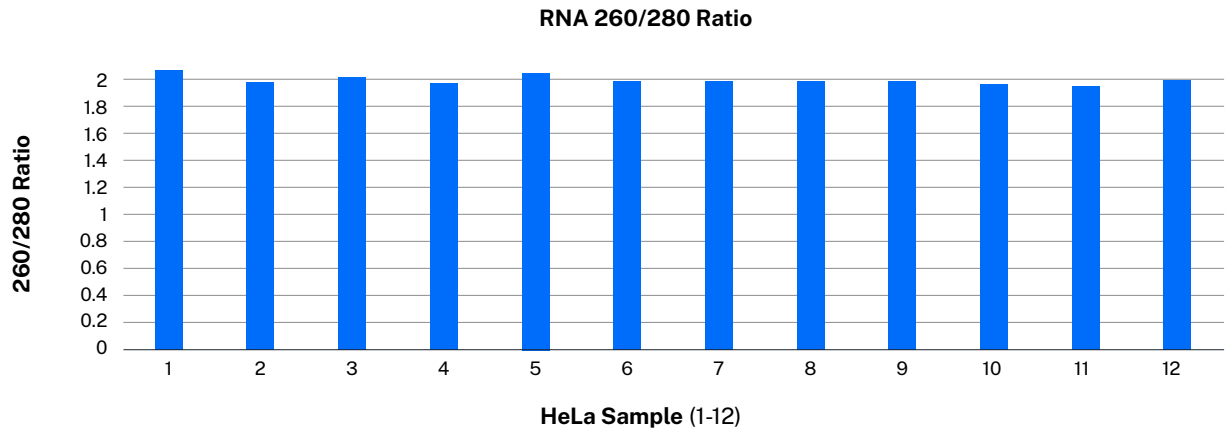


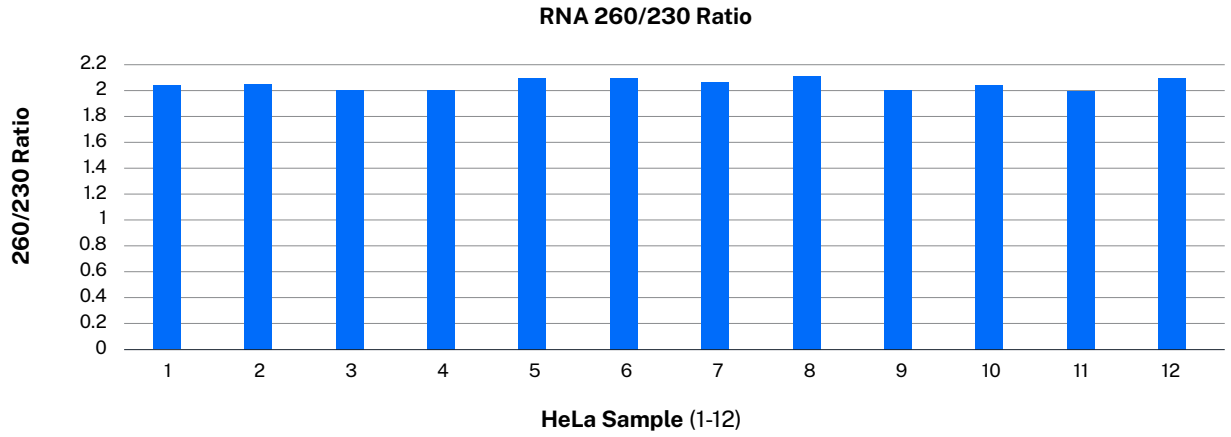
Figure 1. Automated extraction resulted in high and similar concentrations across all samples. RNA from HeLa cells (2.5x10^5 cells/sample) were extracted using the OT-2 Nucleic Acid Extraction Workstation and the Direct-Zol™ kit. The RNA concentration was determined by the NanoDrop™ 2000 UV-Vis Spectrophotometer.

Nucleic acids absorb light at 260nm, while proteins and other contaminates (e.g., phenol, guanidine) absorb light at 280 and 230nm, respectively. RNA purity for the 260/280 and 260/230 absorbance ratios were consistently in line with the expected value of about 2.0 across all samples **(Figure 2A-B)**. All samples showed little RNA degradation as the RIN scores were all above 8.3 (out of a scale of 10) **(Figure 2C)**.

A



B



C

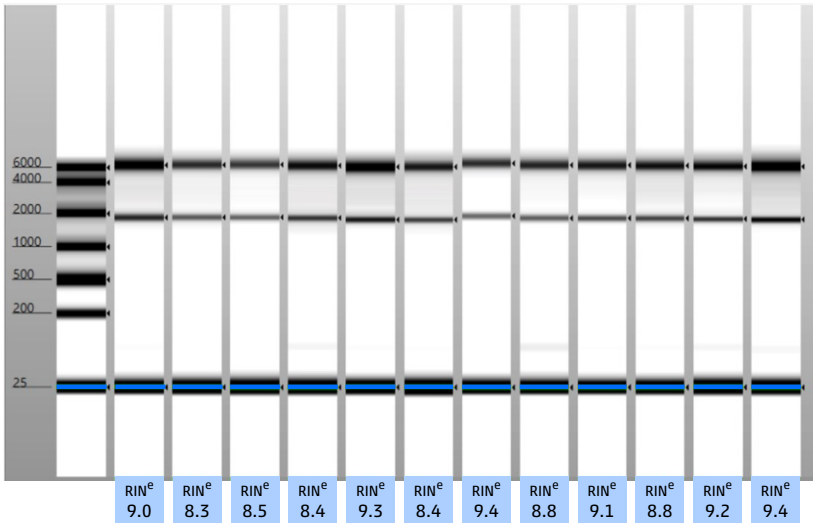


Figure 2. Automated extraction resulted in high purity across all samples. RNA from HeLa cells (2.5x10^5 cells/sample) was extracted using the OT-2 Nucleic Acid Extraction Workstation and the Direct-Zol™ 96 MagBead RNA kit. RNA purity was determined by the NanoDrop™ 2000 UV-Vis Spectrophotometer **(A and B)**. RNA quality and integrity was determined using the using Agilent 2200 TapeStation® **(C)**.

No cross-contamination was present between wells

Wells loaded with DNase/RNase Free-Water did not have detectable RNA (COR, concentration out of range) after the automated extraction was completed **(Figure 3)**. Thus, automation with the OT-2 did not introduce cross-contamination between wells.

CONCLUSION

RNA extraction from HeLa cells was consistent across all samples and therefore illustrates the reproducibility of automation. All extracted RNA samples were of high concentration, purity, and integrity and met expected values. In addition, automation did not introduce cross-contamination. Overall, automating the use of the Direct-Zol kit with OT-2 delivered expected quantity and quality of RNA while saving substantial time and effort. This study demonstrates the value of the OT-2 for nucleic acid extraction and purification used as a starting point in a wide range of applications like transcriptomics, viral detection, miRNA research, cancer research, and more.

RNA Cross Contamination

	1	2	3	4	5	6	7	8	9	10	11	12
A	24.8	COR	25.1	COR	25.8	COR	25.7	COR	27.8	COR	26.9	COR
B	COR	26	COR	27.6	COR	27.1	COR	27.2	COR	27.1	COR	25.7
C	25.6	COR	27.9	COR	26.6	COR	25.8	COR	24.3	COR	25.4	COR
D	COR	26.2	COR	26.4	COR	24.4	COR	27.4	COR	27	COR	28.3
E	27	COR	26.3	COR	26.9	COR	24.5	COR	26.5	COR	27.2	COR
F	COR	26.6	COR	27.1	COR	26.3	COR	25.6	COR	25.9	COR	26.7
G	26.8	COR	25.8	COR	27.2	COR	27.1	COR	26.2	COR	26.8	COR
H	COR	27.4	COR	24.9	COR	25.6	COR	24.6	COR	25.6	COR	26.4

Qubit High Sensitivity Concentration of RNA for both Positive and Negative Control

Positive Control Expected Concentration: ~28 ng/µL

COR = Concentration Out of Range

Figure 3. Cross-contamination between wells during automation was not detected. Extracted RNA and DNase/RNase Free-Water (Negative Control) were added to a 96-well plate in alternating checkerboard fashion. The plate was processed through the Direct-Zol™ 96 MagBead RNA protocol using the OT-2 Nucleic Acid Extraction Workstation. RNA and Negative Controls were quantified by Qubit™ RNA High Sensitivity on the Qubit 3.0 Fluorometer.

REFERENCES

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3. Cai, D., Behrmann, O., Hufert, F., Dame, G. & Urban, G. Direct DNA and RNA detection from large volumes of whole human blood. *Sci. Rep.* 8, 1–9 (2018).