



Automation of the Promega SV 96 Total RNA Isolation System on the Zephyr Genomics Workstation

Introduction

Here, we describe the use of the Zephyr Genomics Workstation in automating total RNA isolation with The Promega SV 96 Total RNA Isolation System (Cat #A3500). Using this system, RNA is purified by binding and eluting to a silica membrane using vacuum filtration. The results confirm the expected quantity and high quality of RNA generated using The Promega SV 96 Total RNA Isolation reagents on the Zephyr Genomics Workstation.

RNA Yield and Quality

Total RNA was harvested from 105 Hela cells/well using Promega SV 96 Total RNA Isolation reagents on the Genomics Workstation. The RNA was eluted in 100 μ L Nuclease-Free water and an average yield of 22.5ng/ μ L was obtained. The purity of RNA was consistently excellent as indicated by the A260/280 ratio. The average RNA Quality Score determined by the LabChip GX software was 9.98.

	Yield (ng/ μ l)	Purity (A260/A280)	RNA Quality Score
Average	22.5	2.2	9.98
%CV	12.2	6.7	1.1

Table 1. Yield and Quality of RNA. RNA purified from the Genomics Workstation were analyzed on the Nanodrop Spectrophotometer and the LabChip GX. The RNA concentration was determined by measuring absorbance at 260nm. The purity was determined by calculating the ratio of the absorbance at 260nm and 280nm. The RNA Quality score was determined by the LabChip GX software.

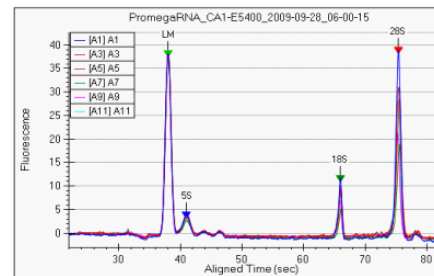


Figure 1. Electropherogram of RNA. RNA purified from the Genomics Workstation were analyzed on the HT RNA assay on the LabChip GX. The overlay of the electropherograms of select samples is shown.

No Cross Contamination Between Wells

The tissue culture cells were seeded into every other well such that cross-contamination could be assessed using a RT-PCR-based assay. Elution from each well were used in RT-PCR reaction to detect cross contamination between wells. cDNA was amplified only from samples that were process from wells containing cells, indicating that contamination of adjacent wells did not occur.

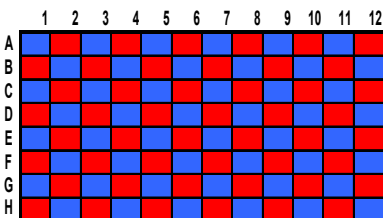


Figure 2. Source pattern of tissue culture cells. 10^5 Hela cells were seeded in each of the blue wells. The red wells were left blank and did not contain any cells.

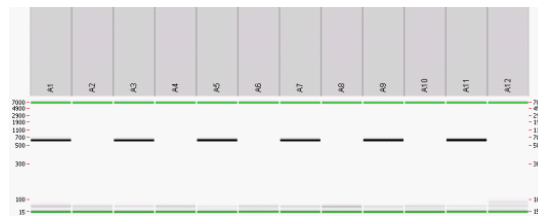


Figure 3. Virtual Gel image of RT-PCR Products. Eluates obtained from samples processed on the Genomics Workstation were subjected to RT-PCR. 1 μ L of eluate were subjected to 35 cycles of RT-PCR using primers specific to GAPDH in a 20 μ L reaction. After thermocycling, the reactions were diluted with 40 μ L of TE buffer and analyzed on the LabChip GX using the HT DNA 5K assay.

Conclusion

Total RNA can be purified in a 96-well format using the Promega SV 96 Total RNA Isolation System in approximately 1 hour. An average yield of 22.5ng/well was obtained. The purity of RNA was consistently excellent as indicated by an average A260/280 ratio of 2.2. The average RNA Quality Score was close to the maximum of 10, indicating fully intact RNA. The RNA is suitable for downstream applications, such as RT-PCR. No cross contamination between wells was detected between wells in a RT-PCR assay. This straightforward and simplified approach to the automation of RNA purification allows for greater throughput, reduces errors and ensures reproducibility.

Zephyr's small foot print fits on to a standard lab bench, requiring no special table, while offering flexibility for molecular biology applications:

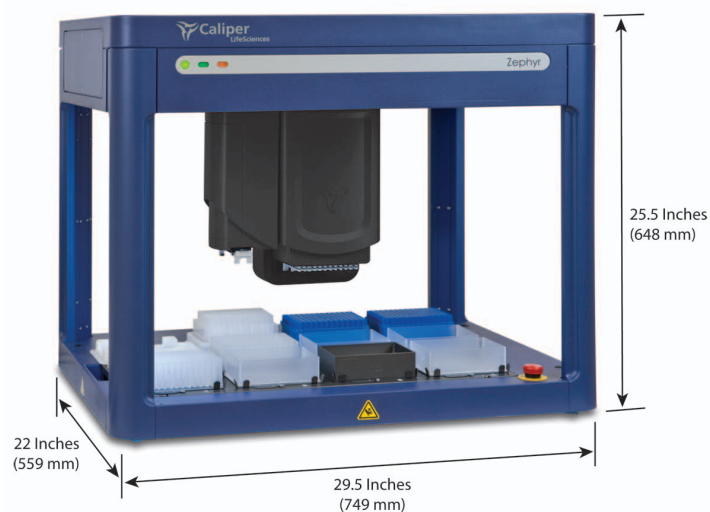
- DNA extraction
- RNA extraction
- Plasmid isolation
- PCR purification
- Sequencing reaction clean-up
- PCR setup
- Sequencing reaction setup
- DNA and RNA normalization

Additional applications which can be automated on the Zephyr Genomics Workstation:

- LabChipGX sample preparation for RNA quality assessment or DNA fragment analysis

What's included with your Zephyr Genomics Workstation

- PC controller and monitor
- Vacuum filtration station and pump
- Waste bottle
- Plate/lid gripper
- Ultrasonic detector
- Genomics Workstation GUI and Maestro software
- Startup Kit



Performance

Volume range:

High Volume Head (HVH) 1 - 200 µL

Precision:

High Volume Head (HVH) 1 - 5 µL: CV <5%
 5 - 200 µL: CV <2%
 2 - 25 µL or 50 µL: CV 5%

Weight:

75 kg (185 lbs) Base Unit

Operating Temperature:

15 - 35 °C (59 - 95 °F)

Operating Humidity:

0 - 85% RH, non condensing

Air Supply:

Regulated 35 - 65 psi

Power Input:

115 VAC, 50/60 Hz, 1000 VA max. or
 230 VAC, 50/60 Hz, 1000 VA max.

Caliper Life Sciences

Part No.	Description
125282	Zephyr Genomics Workstation with HV Head - 110V
125283	Zephyr Genomics Workstation with HV Head - 220V
119728	Optional Zephyr Environmental Enclosure
103263	TurboVap 96 Concentration Workstation
122000	LabChip GX Analyzer

Promega

Product No.	Consumable Kit Name	Qty.
Z3500	SV 96 Total RNA Isolation System	1 Plate
Z3505	SV 96 Total RNA Isolation System	5 Plate

Caliper's Other Genomic Solutions



LabChip GX



TurboVap 96



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