



Efficient, Automated RNA Isolation Using the Ambion MagMAX-96 Total RNA Isolation Kit on the Caliper Life Sciences Sciclone ALH 3000 Workstation

Introduction

Traditional RNA isolation methods typically include difficult-to-automate precipitation protocols or glass fiber filter methods, which can be automated but are prone to problems such as clogging, large elution volume, and inconsistent RNA yield. The Ambion MagMAX magnetic bead-based RNA purification technology provides a fast and simple method for RNA isolation that does not require the use of organic solvents or RNA precipitation, and eliminates problems associated with glass fiber filter-based methods. Magnetic beads provide better RNA binding and higher, more consistent RNA yields. RNA can be eluted in a low volume, 20–50 μL , to concentrate RNA for convenient streamlining of downstream applications. The MagMAX-96 Total RNA Isolation Kit is optimized for high-throughput isolation of RNA from cultured cells, as well as small plant and mammalian tissue samples (up to 10 mg). The protocol is simple and easily adaptable for manual and robotic high-throughput processing.

Materials and Methods

Instrumentation:

Caliper Life Sciences Sciclone ALH 3000 workstation equipped with high volume head, gripper, Z-8, I/O box, and VARIOMAG® On-deck shaker

Reagents:

- MagMAX-96 Total RNA Isolation Kit, (# AM1830)
- 100% Ethanol
- 100% Isopropanol

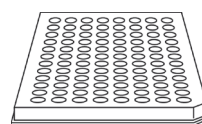
Consumables:

- U bottom plate: provided with the MagMAX-96 Total RNA Isolation Kit.
- 2 mL V bottom plate (Corning, #3960)
- 12 column reservoir (Innovative Microplate, #S30016)
- 150 μL barrier 96 SBS racked pipette tips (MBP, #111426)

Starting Materials:

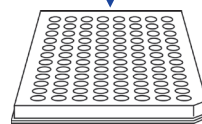
- HeLa and K562 cultured cells

MagMAX-96 Total RNA Isolation Procedure:



Cell Cultures

1. Add Lysis/Binding Solution
2. Add Bead Resuspension Mix
3. Shake for Five Minutes
4. Capture and Wash Beads Twice
5. Treat with DNase (optional)
6. Add Lysis/Binding Solution and Shake for Two Minutes
7. Capture and Wash Beads Two More Times
8. Elute Total RNA



Sample RNA

Results

Figure 1A. Electropherogram

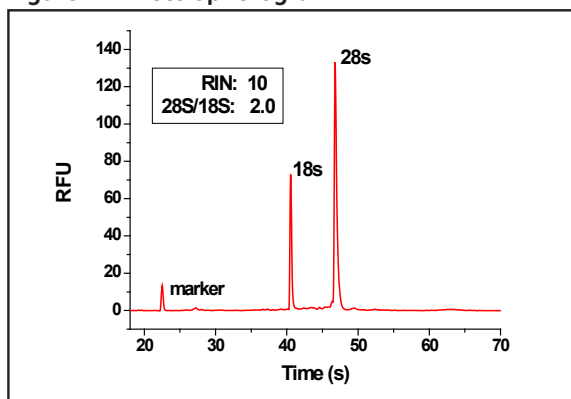


Figure 1B. Real-Time RT-PCR

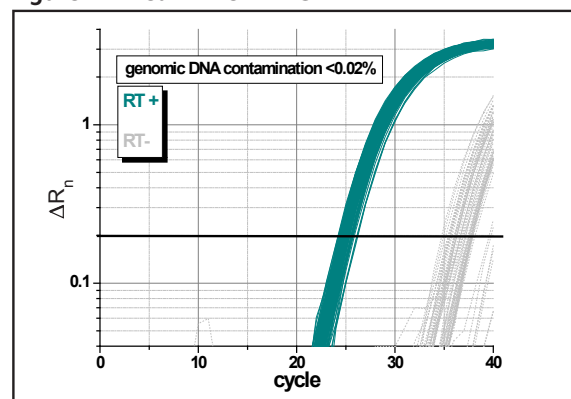


Figure 1. High Quality, High Integrity RNA and Efficient Genomic DNA (gDNA) Removal. **(1A)** RNA of high integrity and quality was isolated from K562 cells using the Ambion MagMAX-96 Total RNA Isolation Kit on the Caliper Life Sciences Sciclone ALH 3000 workstation. Average RNA yield was 28 pg/cell. **(1B)** Efficient gDNA removal was observed. RNA was isolated from HeLa cells (1x10⁴ cells/well) according to the MagMAX Total RNA Isolation protocol on the Caliper Life Sciences Sciclone ALH 3000 workstation and analyzed for RNA recovery and gDNA contamination. gDNA contamination was <0.02% signifying efficient removal.

Figure 2A. Isolated RNA

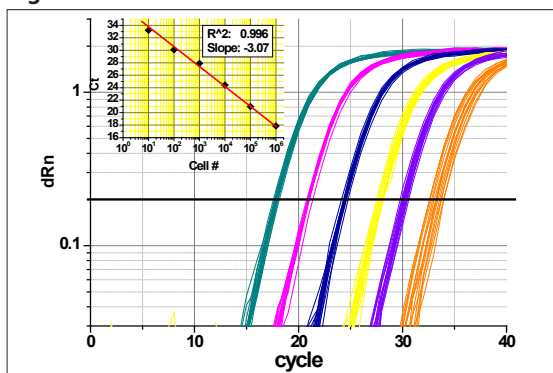


Figure 2B. RNA Yield

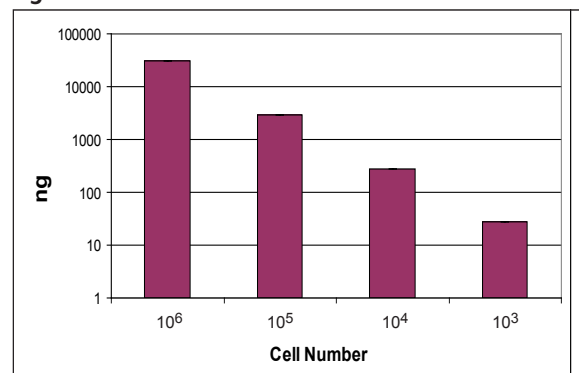


Figure 2. Efficient Recovery of Total RNA from 103–106 Cells Using the Ambion MagMAX-96 Total RNA Isolation Kit. **(2A)** RNA was isolated from 103–106 K562 cells in 16 replicate wells using the Ambion MagMAX-96 Total RNA Isolation Kit. Equivalent volumes of the recovered RNA (4% of eluted volume) were used for qRT-PCR assays (15 μL reaction volume) targeting human RNA Polymerase II mRNA. Recovery of total RNA was linear and highly consistent. **(2B)** RNA yield as assessed by RiboGreen RNA quantitation assays was highly consistent with a coefficient of variance <10% between replicates. RNA yield averaged 28 pg/cell across input amounts.

Conclusion

The Ambion MagMAX-96 Total RNA Isolation Kit provides a fast, simple, and easily automatable method for RNA isolation. The MagMAX RNA binding bead technology selectively binds nucleic acids. This approach results in more consistent RNA recovery and more thorough DNA treatment than glass fiber filter-based RNA methods. This is because the RNA binding beads can be fully re-suspended in solution to enable more thorough mixing, washing, and elution. In addition, magnetic bead-based methods are easier to automate than filter plate-based methods, where vacuum may cause cross-contamination and the occasional filter clog may cause isolation failure of all samples in the whole plate.

In this study we have demonstrated efficient isolation of high quality RNA from cultured K562 cells (Figure 1) with an A260:A280 ribosomal RNA ratio of 2.0 using the Ambion MagMAX-96 Total RNA Isolation Kit on a Caliper Life Sciences Sciclone ALH 3000 workstation. This high quality, high purity RNA is suitable for demanding downstream applications such as quantitative RT-PCR, as seen in Figure 2A. Figure 2B demonstrates the excellent linear recovery of total RNA isolated from different numbers of K562 cells.

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