

# Application Note

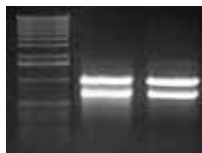
## An Improved Method to Isolate Inhibitor-Free RNA from Turbid and Non-turbid Water Samples

Heather Callahan, Ph.D.

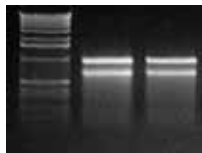
MO BIO Laboratories, Inc., 2746 Loker Avenue West, Carlsbad, CA 92010

### Introduction

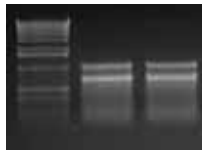
Traditional purification of RNA from water samples relies almost exclusively on methods that employ the use of hazardous chemicals such as phenol as well as time consuming precipitation, washing and resuspension steps. In addition, the purchase and use of DNase I after RNA purification is required to obtain pure RNA samples. Despite the time and effort involved, inhibitors of downstream applications such as reverse transcription, RT-PCR, and qRT-PCR are often not removed. The PowerWater® RNA Isolation Kit, developed by MO BIO Laboratories, is a complete system to isolate intact RNA from all types of filter membranes that is both inhibitor and DNA free.



**Figure 1.** Total RNA isolated from water samples vacuum filtered in duplicate through 0.22 µm polyethersulfone membranes. Top band is 23S rRNA. Lower band is 16S rRNA



**Top:** Water spiked with 2 ml of an overnight *E. coli* culture



**Middle:** Water spiked with 2 ml of an overnight *E. faecalis* culture

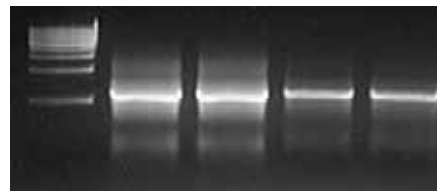
**Bottom:** 50 ml of an inhibitor-rich lagoon sample.

### Optimized Protocol

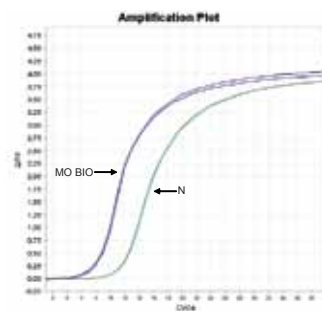
The PowerWater® RNA Isolation Kit has been optimized for use with water samples filtered through all types of membranes. The kit utilizes our novel PowerWater® bead tube for optimal lysis and our patented Inhibitor Removal Technology® (IRT) for the removal of suspended solids and dissolved compounds. In addition, the kit includes RNase free DNase I for on-spin column DNA removal which results in not only faster DNA removal but more efficient removal of the DNase enzyme itself. The kit was used to isolate RNA from non-turbid water spiked with Gram – and Gram + organisms as well as microbial RNA from water collected from a turbid, inhibitor rich lagoon (**Figure 1**).

### Successful Endpoint and Real Time RT-PCR

Both endpoint and real-time RT-PCR was used to assess inhibitor removal and RNA quality. Purified RNA was quantified using a NanoDrop, then diluted to the same concentration and reverse transcribed, followed by PCR with two different sets of primers. For endpoint PCR a weaker RT-PCR product was generated using a competitor's kit (N) suggesting carryover of contaminants (**Figure 2**). For real-time PCR, a 4 cycle difference in amplification occurred between the PowerWater® kit (blue) and the Competitor N kit (Green) suggesting a reduction in detection levels not related to initial RNA concentration (**Figure 3**).



**Figure 2.** Two-step RT-PCR results from total RNA isolated from inhibitor rich lagoon water (Carlsbad, CA). 30 ml was vacuum filtered in duplicate through 0.22 µm polyethersulfone membranes (PowerWater®) or proprietary membranes (Competitor N). 1 µl of each RT reaction was used as template. Universal 16S rDNA primers were used to generate a 1,243 bp product.



**Figure 3.** Two-step Real-Time RT-PCR results for RNA isolated from inhibitor rich lagoon water (Carlsbad, CA) using the MO BIO kits (blue) and Competitor N (green) kits. 1 µl of each RT reaction was used as template. Assay efficiency was 104%. Primers were specific for the 16S rRNA gene. NTC controls amplified at a Ct = 27 (curve not shown).

### Summary

RNA isolation from filtered water samples usually involves hazardous chemicals or the use of commercial kits that have not been designed for use with filter membranes. The PowerWater® RNA Isolation Kit has not only been optimized for use with all types of filter membranes but the streamlined protocol includes both patented IRT and RNase free DNase I for on-spin column DNA removal. This results in a complete kit with an easy to follow protocol yielding consistent, quality RNA that can be used successfully in downstream applications.