

# Application Forum

## Improved DNA and RNA isolation from biofilms

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### Introduction

Biofilms are composed of bacteria irreversibly attached to a substrate by extracellular polymeric substances or EPS. Within this EPS matrix, a number of compounds can be found including humic substances, metals, salts, and pesticides. As a result, microbes within a biofilm are difficult to lyse and the nucleic acids, once purified, may still contain inhibitory substances. The PowerBiofilm™ DNA Isolation Kit and PowerBiofilm™ RNA Isolation Kit from MO BIO Laboratories combine biofilm pretreatment and improved cell lysis with patented Inhibitor Removal Technology® to yield consistent, high-quality, inhibitor-free DNA and RNA.

### EPS removal and lysis optimization

One of the most difficult aspects of nucleic acid isolation and purification from biofilms is ensuring complete lysis of the microbial community in the presence of EPS. EPS degradation can be achieved a number of ways using chemical, mechanical, or enzymatic means. The PowerBiofilm™ kits use a combination of several methods to dissolve the EPS, which in turn fully exposes the microbes to the lysis buffers (Figure 1, A and B).

### Inhibitor removal

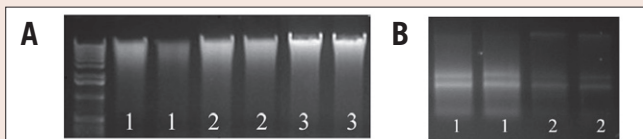
Even with efficient lysis, degraded EPS and other organic/inorganic compounds can carry over through purification and inhibit downstream applications of nucleic acids. To prevent this, both the PowerBiofilm™ DNA and PowerBiofilm™ RNA Isolation Kits contain patented Inhibitor Removal Technology® (IRT), which has been shown to remove humic substances, polysaccharides, and polyphenolics from nucleic acid preps (www.mobio.com/references).

### Sample validation

Biofilms occur virtually everywhere and are as diverse as the microbes that create them. Therefore, a wide range of biofilm types have been evaluated both at MO BIO and by outside collaborators (Table 1, A and B). High DNA and RNA yielding biofilms were tested as well as low yielding microbial mats.

### Summary

The PowerBiofilm™ DNA and PowerBiofilm™ RNA Isolation Kits are the first of their kind developed to isolate high-quality,



**Figure 1. Genomic DNA and RNA isolated from biofilms.** (A) Genomic DNA isolated from 0.15 g of phototrophic mat during PowerBiofilm™ DNA Isolation Kit development. 1, standard glass bead tube mix; 2, PowerBiofilm bead tube mix; 3, PowerBiofilm bead tube mix with BF2 (lysis enhancement buffer). Variation 3 represents the final chemistry of the kit. (B) Total RNA isolated from 0.15 g of an inhibitor rich lagoon biofilm sample using the PowerBiofilm™ RNA Isolation Kit. 1, PowerBiofilm™ bead tube mix with BFR2 (lysis enhancement buffer); 2, Standard glass bead tube mix without BFR2.

Biofilm Type	Sample Amount (g)	DNA Yield (ng/μl)	Data
Sink Pipe	0.20	94 - 198	
Lagoon Rocks	0.15	100 - 150	
Phototrophic Biofilm (Microbial Mat)	0.15	54 - 13	
	0.10	70 - 76	
	0.05	37 - 50	
Stream Rocks	<0.05	4 - 11	Data courtesy of A. J. Gildemeister University of Sterling
Bioreactor	0.25	56 - 130	Data courtesy of J. Moore-Kucera Texas Tech University
Button Thrombolites (Microbial Mat)	0.25	1 - 15	
Samples courtesy of J. Foster University of Florida			
Gypsum Crust	0.20	15 - 28	Data courtesy of B. Camara & A. Gorbushina Federal Institute for Material Research and Testing Berlin, Germany

Biofilm Type	Sample Amount (g)	RNA Yield (ng/μl)	Data
Lagoon Rocks	0.20	37 - 60	
Phototrophic Biofilm (Microbial Mat)	0.10 - 0.15	10 - 100	
Button Thrombolites (Microbial Mat)	0.25	1 - 17	Data courtesy of J. Foster University of Florida
Deep Sea (Microbial Mat)	0.20 - 0.30	11 - 35	
Data courtesy of P. D. Countway and D. A. Caron University of Southern California			

**Table 1. (A) PowerBiofilm™ DNA and (B) PowerBiofilm™ RNA Isolation Kit yields from biofilm and microbial mats.** Data was generated by both MO BIO Laboratories and collaborators as indicated.

inhibitor-free DNA and RNA from biofilm samples including microbial mats. Sample pretreatment combined with a novel lysis mix and our patented Inhibitor Removal Technology® results in optimal nucleic acid yields that are free of inhibitors and ready to be used in all downstream applications.

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