

Magnetic bead-based solution for DNA extraction from environmental samples using Mag-Bind® Environmental DNA 96 Kit from Omega Bio-tek

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Introduction

The study of environmental metagenomics is key in unraveling the complex diversity of the microorganisms that inhabit different ecosystems and their contribution to the key characteristics of these ecosystems. The outcome of these studies has several practical implications and can impact several different fields. Some of the applications include water quality assessments, agricultural microbiome analysis studies to improve soil quality, crop production, ecological remediation and other biological investigations on environmental samples etc. Nucleic acid extraction is the first crucial step and sufficient amounts of high-quality nucleic acids representative of all the organisms present in a given environmental sample must be obtained for any comprehensive downstream analysis. Environmental samples are particularly challenging owing to the presence of naturally occurring inhibitors such as humic acid, fulvic acid and other organic matter that can co-purify along with the DNA potentially interfering with sensitive downstream applications. Omega Bio-tek's Mag-Bind® Environmental DNA 96 Kit (M5645) was developed to isolate high quality DNA from various environmental samples such as soil, water etc and uses a proprietary inhibitor removal reagent (cHTR) to eliminate inhibitors from the extraction process. The kit uses magnetic beads for purification making it amenable for high-throughput processing on various open-ended liquid handlers or magnetic processors. We validated the Mag-Bind® Environmental DNA 96 Kit on soil and water samples and compared its performance with Company Q's soil and water DNA isolation kits respectively. The kit performance was evaluated in terms of yield and quality of the DNA obtained as well as sensitivity of real-time PCR amplification downstream.

Materials and Methods

Soil DNA extraction

Outdoor soil (250 mg) was spiked with approximately 1 mL culture of *Bacillus subtilis* and DNA was extracted using Mag-Bind® Environmental DNA 96 Kit (Omega Bio-tek) and Soil DNA Isolation kit (Company Q) following manufacturer's recommended protocols. The isolations were carried out in quadruplicate for the Omega kit and in duplicate for the competitor's kit.

Water DNA extraction

Pond water (100 mL) was spiked with approximately 1 mL culture each of *B. subtilis* and *E. coli* harboring pGEM plasmid and was filtered through Sterivex (Millipore SVGPL10RC) filters. Upon filtration, DNA was extracted from these filters in quadruplicate following manufacturer's recommended protocols.

DNA Quantification and Quality Assessment

Following the soil and water DNA extraction protocols, purified DNA was eluted in 100 µL elution buffer included in each kit and quantified using Thermo Scientific's NanoDrop® 2000c. The quality of the purified DNA was analyzed by performing real-time PCR using *B. subtilis* specific primers on undiluted and 10-fold diluted DNA isolated from soil extraction protocol and on 10-fold and 100-fold diluted DNA isolated from water extraction protocol. Briefly, a qPCR reaction was set up to a total volume of 20 µL using Agilent's Brilliant III 2X SYBR® as the master mix and 2 µL of template DNA at appropriate dilutions amplified with suitable primers following a standard protocol on the ABI 7900.

Results and Discussion

The DNA yields and absorbance ratios (A260/A280 and A260/A230) from the soil samples spiked with *B. subtilis* cells using the Omega kit and Company Q's kit are as shown in Table 1. The average DNA yield from the Omega kit was ~3.03 µg compared to 1.4 µg from Company Q's kit corresponding to a 116% increase in yield using the Omega kit. The average A260/A280 and A260/A230 ratios using Omega Bio-tek's kit were better compared to Company Q's. The A260/A280 ratios were 1.86 ± 0.03 (Omega) and 1.37 ± 0.05 (Company Q) and A260/A230 ratios were 1.13 ± 0.07 (Omega) and 0.65 ± 0.05 (Company Q) (Table 1). These ratios suggest superior DNA quality post extraction using Omega Bio-tek's kit when compared to Company Q's kit.

Soil	Yield (µg)	A260/A280	A260/A230
Omega Bio-tek	3.16	1.86	1.11
	3.15	1.84	1.21
	2.92	1.9	1.08
	2.9	1.85	1.2
Company Q	1.4	1.33	0.58
	1.4	1.4	0.65

Table 1. DNA yields and absorbance ratios using kits from Omega Bio-tek and Company Q following soil protocol.

Table 2 shows the yield and absorbance ratios obtained using Omega Bio-tek and Company Q's kits following extraction with the water protocol. The average yields with the Omega kit are significantly higher, 6.07 µg vs 3.2 µg ($p < 0.05$; Tukey's post-hoc analysis). The A260/A280 ratios were comparable for the two kits; however, the A260/A230 ratios were better with the Omega kit, 1.87 vs 1.44 suggesting lower inhibitor carryover.

Water Protocol	Yield (µg)	A260/A280	A260/A230
Omega Bio-tek	5.71	1.77	1.84
	6.8	1.78	1.88
	5.98	1.8	1.84
	5.78	1.8	1.9
Company Q	2.9	1.86	1.36
	3.51	1.89	1.33
	3.02	1.87	1.09
	3.38	1.87	1.95

Table 2. DNA yields and absorbance ratios using kits from Omega Bio-tek and Company Q following water protocol.

The quality of the DNA obtained from each extraction following soil and water protocols was also assessed by comparing Ct values generated from a qPCR reaction. Figure 1 shows the average Ct values obtained at different dilutions of the purified DNA when amplified using *B. subtilis* specific primers. For extractions following the soil protocol, there was no detectable amplification in the undiluted DNA extracted using the kit from Company Q suggesting inhibitor carryover from the sample to the eluted DNA. In contrast, DNA purified using Omega Bio-tek's kit exhibited good amplification potential at undiluted levels corroborating the efficacy of its proprietary inhibitor removal reagent employed during the extraction process. The Ct values at 10X dilution were lower by 1.3 cycles using the Omega kit indicating a 2.46-fold DNA yield difference between the Omega and Company Q extraction supporting the yield difference when quantified using NanoDrop.

Average Ct values at different dilutions

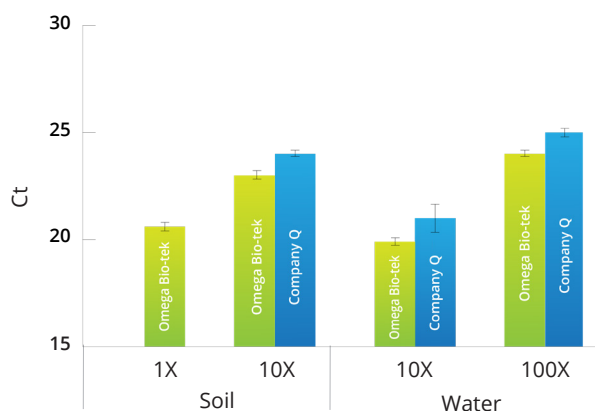


Figure 1. Average Ct values obtained after amplifying the purified DNA from Omega Bio-tek and Company Q's kits with *B. subtilis* specific primers following soil and water protocols.

For extractions following the water protocol, the Ct values at 10X and 100X dilutions were lower with Omega extraction compared to Company Q extraction supporting the higher DNA yields with the Omega kit. The Δ Ct between the serial dilutions were ~3.3 for the Omega kit and ~4.3 for the Company Q kit. This data suggests that Omega Bio-tek's kit performed as well as the Company Q kit in eliminating PCR inhibitors from the isolations but with superior yields.

Conclusions

Omega Bio-tek's Mag-Bind® Environmental DNA 96 Kit performed significantly better than the Company Q's kits designed for soil and water extractions both in terms of DNA yield as well as quality. The Omega kit was more effective in removing the PCR inhibitors from the soil sample compared to the Company Q and was comparable when it came to inhibitor removal from the water sample. Omega-isolated DNA amplified consistently sooner than the Company Q-isolated DNA represented by their lower Ct values at different dilutions indicating higher DNA yields. The Company Q's kits are column-based whereas the Omega Bio-tek's kit utilizes magnetic beads for purification. This is an added advantage with the Omega kit making it automation-friendly, providing a rapid and reliable high throughput solution. Overall, the results show that the Omega Bio-tek's Mag-Bind® Environmental DNA 96 Kit isolates high yielding, high quality DNA free of inhibitors compatible with various downstream applications such as qPCR, next-generation sequencing etc.

Product Information

Product No.	Description
M5645-00	Mag-Bind® Environmental DNA 96 Kit (1 x 96 Preps)
M5645-01	Mag-Bind® Environmental DNA 96 Kit (4 x 96 Preps)