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Modified DNeasy PowerWater Kit® protocol for DNA extractions from drinking water samples

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ABSTRACT

DNA-extractions from drinking water samples are essential for a range of subsequent microbial community quantitation and characterization methods, i.e., quantitative polymerase chain reaction (qPCR) assays targeting specific genes and the characterization of compositional and functional profiles using high throughout sequencing technologies (e.g. amplicon sequencing and shotgun metagenomic sequencing). Despite advances in the specificity and sensitivity of molecular techniques, efficient recovery of DNA from drinking water samples, particularly those with low cell counts, remains challenging. Drinking water samples, in which microbial concentrations range between $10^3$and 10^5 cells.ml^{-1}, generally requires the collection of large volume of sample and subsequent processing by filtration to concentrate microbial cells. Here we document a modified version of the DNeasy PowerWater Kit® protocol that utilizes enzymatic, chemical, and mechanical lysis strategies to enhance recovery of DNA from drinking water samples. The DNA quantities recovered using this protocol are typically at least two to three-fold higher when compared to the routine DNeasy PowerWater Kit® protocol. In our hands, this protocol consistently provides sufficient DNA of high quality from as little as 1.5 liters of filtered drinking water with cell counts in the range of 10^3-10^4 cells.ml^{-1}, while maintaining the 16S rRNA qPCR counts at least 100-1000 times higher compared to DNA extracts from negative controls (i.e., blank unused filters, filters with autoclaved deionized water filtered, and reagent blanks) processed identically as the drinking water samples.

MATERIALS

NAME	CATALOG #	VENDOR
Lysing Matrix E	116914050-CF	MP Biomedicals
Chloroform/Isoamyl Alcohol (24:1)	327155000	Acros Organics
Tris-EDTA (10X)	786-033	G-Biosciences
Lysozyme Solution (50 mg/mL)	90082	Thermo Fisher Scientific
Proteinase K Solution (20 mg/mL)	AM2546	Thermo Fisher Scientific
DNeasy PowerWater Kit	14900-50-NF	Qiagen
Sterivex-GP Pressure Filter Unit	SVGP01050	Emd Millipore
DNA LoBind Microcentrifuge Tubes	022431021	Eppendorf
Eppendorf™ 5424 Microcentrifuge	05400002	Eppendorf
Eppendorf™ ThermoMixer C Bundle	2231000574	Eppendorf
FastPrep-24™ Classic Instrument	116004500	MP Biomedicals
Fisherbrand™ Variable Speed Mini Vortex Mixer	14955163	Fisher Scientific
Fisherbrand™ Fine Point High Precision Forceps	22327379	Fisher Scientific
Fisherbrand™ High Precision Metal Scalpels	08-920B	Fisher Scientific
Petri Dish with Clear Lid	FB0875712	Fisher Scientific

NAME	CATALOG #	VENDOR
QIAcube System	9001882	Qiagen

SAFETY WARNINGS

Chloroform/isoamyl alcohol treatment should be performed in a laminar fume hood certified for the use of volatile organics. Please refer to the SDS of chloroform/isoamyl alcohol before using it: **Chloroform:isoamyl alcohol SDS.PDF**

BEFORE STARTING

- Aseptically transfer the ceramic spheres, silica spheres, and glass bead contained in the 2 ml Lysing Matrix E tube (MP Biomedicals, Cat. No: 116914100) to a corresponding 1.5 ml microcentrifuge tubes (Eppendorf, Cat. No: 022431021). Exclusion of these components, essential for downstream mechanical treatment (i.e. bead beating), ensures that the processed polyethersulfone (PES) membrane extracted from filter unit is fully immersed in solution during enzymatic and chemical treatment (STEPS 2 and 3, respectively).

Important points to address before start, as listed in the DNeasy PowerWater Kit Handbook:

- Solution PW1 must be warmed at **55 °C** for **00:05:00** – **00:10:00** min to dissolve precipitates prior to use. Solution PW1 should be used while still warm.
- If Solution PW3 has precipitated, heat at **55 °C** for **00:05:00** – **00:10:00** to dissolve precipitate.
- Shake to mix Solution PW4 before use.

PROCESSING OF THE STERIVEX-GP PRESSURE FILTER UNIT

1 On the surface of a sterile petri dish (Fisher Scientific, Cat. No: FB0875712), cut the PES filter membrane contained in the Sterivex-GP Pressure Filter Unit (EMD Millipore, Cat. No: SVGP01050) into smaller pieces using a sterile scalpel (Fisher Scientific, Cat. No: 08-920B), and subsequently transfer the cuttings into the 2 ml Lysing Matrix E tube (MP Biomedicals, Cat. No: 116914100) using sterile tweezers (Fisher Scientific, Cat. No: 22327379). For details on how to extract the filter from the Sterivex-GP Pressure Filter Unit, please refer to the following video using this link: https://www.dropbox.com/s/m1ccznfsp02gy2n/IMG_5384.MOV?dl=0

Before handling the Sterivex-GP Pressure Filter Unit (EMD Millipore, Cat. No: SVGP01050), aseptically transfer the ceramic spheres, silica spheres, and glass bead contained in the 2 ml Lysing Matrix E tube (MP Biomedicals, Cat. No: 116914100) to a corresponding 1.5 ml microcentrifuge tubes (Eppendorf, Cat. No: 022431021). Exclusion of these components, essential for downstream mechanical treatment (i.e. bead beating), ensures that the processed polyethersulfone (PES) membrane extracted from filter unit is fully immersed in solution during enzymatic and chemical treatment (STEPS 2 and 3, respectively).

ENZYMATIC TREATMENT WITH LYSOZYME

2 Add **294 µl** 10X Tris-EDTA (100 mM Tris, 10 mM EDTA, pH 8.0, G-Biosciences, Cat. No: 501035446) supplemented with 6 µl lysozyme solution (50 $mg.ml^{-1}$, Thermo Fisher Scientific, Cat. No: 90082) to the 2 ml Lysing Matrix E Tube. Vortex and then incubate for **60:00:00** min at **37 °C** with light mixing at **300 rpm** using the Eppendorf ThermoMixer C (Eppendorf, Cat. No: 5382000015) making sure that the filter membrane pieces are fully immersed in the solution.

Final concentration of lysozyme in **300 µl** solution: 1 $mg.ml^{-1}$

CHEMICAL TREATMENT AND THE ADDITION OF PROTEINASE K

3 Add **300 µl** PW1 - provided in the DNeasy PowerWater kit and **30 µl** Proteinase K (20 $mg.ml^{-1}$, Thermo Fisher Scientific, Cat. No: AM2546). Vortex and then incubate for **30:00:00** min at **56 °C** with light mixing at **300 rpm** using the Eppendorf ThermoMixer C (Eppendorf, Cat. No: 5382000015) making sure that the filter membrane pieces are fully immersed in the solution.

Final concentration of Proteinase K in **600 µl** solution: 1 $mg.ml^{-1}$

CHLOROFORM/ISOAMYL ALCOHOL AND MECHANICAL TREATMENT (BEAD BEATING)

4 Aseptically transfer the ceramic spheres, silica spheres, and glass bead contained in the 1.5 ml microcentrifuge tubes (Eppendorf, Cat. No: 022431021) to corresponding 2 ml Lysing Matrix E tube (MP Biomedicals, Cat. No: 116914100).

5 Add **630 µl** chloroform/isoamyl alcohol 24:1 (Acros Organics, Cat. No: 327155000) and bead beat at setting 6 for **00:00:40** sec using the FastPrep -24™ Classic Instrument (MP Biomedicals, Cat. No: 116004500).

Chloroform/isoamyl alcohol treatment should be performed in a laminar fume hood certified for the use of volatile organics. Please refer to the SDS of chloroform/isoamyl alcohol before using it: **Chloroform:isoamyl alcohol SDS.PDF**

6 Centrifuge for **00:10:00** min at **14000 x g** at **4 °C** and transfer the upper aqueous phase to a clean 1.5 ml microcentrifuge tubes (Eppendorf, Cat. No: 022431021).

Expected supernatant recovery range between **600 µl** and **630 µl**

DNA EXTRACTION USING THE QIACUBE SYSTEM OR QIAGEN HANDBOOK

7 Use **600 µl** of the recovered aqueous phase as sample for the QIACube System (QIAGEN, Cat. No: 9001882). If the sample is less than **600 µl** add solution PW1 up to the final volume. Follow the instructions as indicated in the *DNeasy PowerWater Kit QiaCube Protocol Sheet*. If extractions are preformed manually, please continue with steps 11 to 23 as described in the *DNeasy PowerWater Kit Handbook.*

DNeasy PowerWater Kit QiaCube Protocol Sheet.pdf

DNeasy PowerWater Kit Handbook.pdf

8 Store the DNA at **-80 °C** until further processing.