Apr 19, 2024

DNA extraction of water sample from sylphium filters

DOI
dx.doi.org/10.17504/protocols.io.4r3l22y83l1y/v1

Omneya Osman[1]

[1]IVL Swedish research Institute

Omneya Osman
IVL Swedish research Institute

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.4r3l22y83l1y/v1

Protocol Citation: Omneya Osman 2024. DNA extraction of water sample from sylphium filters. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l22y83l1y/v1

Protocol status: Working
We use this protocol and it's working

Created: November 06, 2023

Last Modified: April 19, 2024

Protocol Integer ID: 90491

Abstract

https://www.qiagen.com/se/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-blood-and-tissue-kit

Guidelines

- Nucleic acid must be extracted and handled in a second designated area, using a separate set of pipettes, filter tips, tube racks, fresh gloves, lab coats and other equipment.
- Amplified products must not be pipetted in this area.
- Samples should be stored in designated fridges or freezers in the same area.

Materials

https://sylphium.com/webshop/product/syl010/

Before start

- The workspace should be cleaned with bleach solution and ethanol.

Prepare lysate

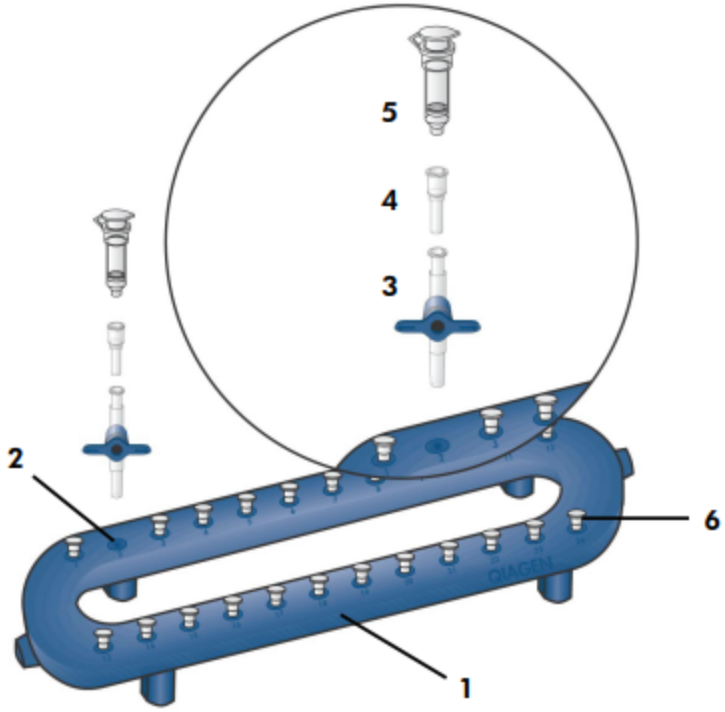
1
- Prepare sampling kit (sylphium filter, gloves, ATL buffer, syringe)
- Find an interesting of environmental site.
- Inject 1-3 L of environmental water into sylphium filter using a clean sterile beaker and 50 ml syringe.
- Inject a volume of 3 ml ATL buffer (Qiagen) in the sylphium filter to be stored at room temperature until it is transferred and processed in the laboratory.
- Sylphium filters were stored at 4 °C once they arrived to laboratory.
- Incubate Sylphium filter at 56°C for 30 min to dissolve any crystalls of ATL buffer.
- Transfer the buffer from the sylphium filter capsule using 3 ml syringe into a 15 mL falcon tube.
- Be careful not to apply too much pressure when collecting lysate from sylphium filter.
- The volume of lysate might vary between 2-3 ml.
- Add proteinase K with the ratio 10 µl for 100 µl lysate, Vortex for a few seconds and incubate at 56°C overnight.

DNA extraction from the lysate

2 Remove the lysate from the incubator and add equal volume of AL buffer (Qiagen), Vortex for a few seconds and incubate at 56°C for 10 min.

3 Add equal volume of 99.9% cold ethanol Vortex briefly,
The ratio of sample lysate: AL buffer: Ethanol =1:1:1.

4 Place DNeasy Mini spin column in a vacuum manifold and load the above mixture (5-15 ml) in the tube extender tube connected to the spin column and apply the vacuum for lysate flow through the spin column as shown below.



1. QIAvac 24 Plus vacuum manifold
2. Luer slot of the QIAvac 24 Plus
3. VacValves*
4. VacConnector*
5. QIAamp Mini spin column
6. Luer slot closed with luer plug

5

Remove the DNeasy min spin column from the vacuum fold
Place it in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at 6000 g or (8,000 rpm).
Discard flow-through and collection tube.

6

Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 1 min at 20,000 ∗ g (14,000 rpm) t. Discard flow-through and collection tube.

Place spin column in a new collection tube, centrifuge for 2 min at 17,000 ∗ g (13,000 rpm) to dry the DNeasy membrane from ethanol residual.

7

Transfer the spin column to a new 1.5- or 2-mL DNA LoBind tube with caps removed.

Add 100 µl 70°C Buffer TE (pH 8.0) to the membrane and incubate at room temperature for 10 min.

Centrifuge for 1 min at 6,000 ∗ g (8,000 rpm) to collect the DNA in 1.5- or 2-mL DNA LoBind tube and discard the spin column.

8 Quantify the DNA extract with nanodrop photometer to evaluate the quality of extracted DNA.