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# DNA Extraction - Qiagen DNeasy Blood and Tissue Kit UCC & IMR eWHALE

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

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## Abstract

This DNA extraction protocol was used in Rodriguez et al., 2024 (in prep) for the extraction of environmental DNA from samples collected off of the coast of Ireland (Atlantic) and Norway (Norwegian Sea) as a part of the eWHALE project. Work was carried out by researchers at University College Cork (UCC) and the Institute for Marine Research (IMR). The protocol outlines lysis from Sterivex (0.45 µm pore size; Merck Millipore ID: SVHV010RS) eDNA filters and DNA extraction of lysates using the Qiagen DNeasy Blood and Tissue Kit. Mostly, the protocol follows the official version with spin columns published by Qiagen for the DNeasy Blood and Tissue Kit (available here: https://www.qiagen.com/us/resources/resourcedetail?id=68f29296-5a9f-40fa-8b3d-1c148d0b3030&lang=en), however, researchers at IMR used a QiaVAC 24 Plus vacuum system (ID: 19413, Qiagen) rather than centrifugation for spin column steps.

## Guidelines

Lysis should be done in a laminar flow hood.
Always change pipette tips while working with different lysates.
All centrifugation steps should be carried out at room temperature (15–25°C).
All reagents in the DNeasy extraction kit are viable for 1 year under the appropriate conditions (kept in a dry space at room temperature).
If Buffer AL precipitates at any point, warm to 56°C until the precipitate disolves.

## Materials

- DNeasy Blood & Tissue Kit (ID for 50 preps: 69504 50, Qiagen)
- Pipettes : monochannel p10, p100, p1000, p5000 and corresponding filter tips
- 1.5 mL and 2 mL microcentrifuge tubes
- Ethanol (96-100%)

## Safety warnings

Always use gloves and perform work in a room with adequate ventilation.
DNeasy buffers will react with bleach - safely dispose of these chemicals.

## Ethics statement

Not applicable.

## Before start

Add the appropriate amount of ethanol (96-100%; required volume is listed on the label of each bottle) to Buffers AW1/AW2 if necessary (they are included in the kit as concentrates and need to be diluted).

## Lysis

3h 45m

1 Remove filters from the freezer and allow to thaw at room temperature for 00:45:00 . Ensure that all filter capsules are properly closed and labeled.

45m

2 Invert filters a couple of times to agitate lysate.

*

3 Incubate filter capsules at 56 °C for 03:00:00 . Invert filters every 30 minutes.

3h

4 Label 2 mL screw cap tubes or microcentrifuge tubes for all lysates .

5 Using a 2 or 3 mL syringe, evacuate the lysis buffer from the filter capsule by pushing in 1 mL of air (while holding the filter horizontally) then evacuating the lysis buffer (while holding the filter vertically).

6 Add the lysates from the syringe (0.5 - 2 mL) to the associated labeled tube.

## Extraction

7m 30s

7 After incubation, vortex tubes for 00:00:15 then add 200 µL Buffer AL.

15s

8 Vortex again for 00:00:15 then add 200 µL ethanol (96-100%).

15s

9 Pipette the mixture (including any precipitate) into a labeled DNeasy Mini spin column placed in a 2 mL collection tube.

10 Centrifuge 6000 x g, 00:01:00

1m

11 Discard the flow-through + collection tube.

12 Place the spin column into a new 2 mL collection tube and pipette 500 µL of Buffer AW1 from the DNeasy kit into the column.

13 Centrifuge 6000 x g, 00:01:00 1m

14 Discard the flow-through + collection tube.

15 Place the spin column into a new 2 mL collection tube and pipette 500 µL of Buffer AW2 from the DNeasy kit into the column.

16 Centrifuge 20000 x g, 00:03:00 3m

17 Discard the flow-through + collection tube.

> **Note**
>
> Make sure that the DNeasy spin column membrane is dry after this step since residual ethanol may interfere with subsequent reactions.

18 Remove the spin column carefully from the collection tube (do not allow it to come into contact with flow-through).

19 Place the spin column into a clean 1.5 or 2 mL microcentrifuge tube and pipette 200 µL Buffer AE onto the spin column membrane.

20 Incubate the samples at room temperature for 00:01:00 . 1m

21 Centrifuge 6000 x g, 00:01:00 to elute 1m

22 Repeat the previous 2 steps (incubate + centrifuge).

Note

Do not elute more than 200 µL into a 1.5 mL microcentrifuge tube because the DNeasy spin column will come into contact with the eluate.

## Protocol references

Detailed guidelines and ordering information can be found on the official product webpage: https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-blood-and-tissue-kit