Sep 03, 2024

Environmental DNA (eDNA) Extraction at OSU using the KingFisher Flex with the Omega Bio-Tek Mag-Bind® Blood & Tissue DNA HDQ 96 Kit

Forked from DNA EXTRACTION Protocol Template

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Better Biomolecular Ocea…

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

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Abstract

This protocol describes the DNA extraction process at Oregon State University (OSU) using the KingFisher Flex with the Omega Bio-Tek Mag-Bind Blood & Tissue DNA HDQ 96 Kit. Specific modifications to the original protocol are bolded for easy identification.

MIOP: Minimum Information about an Omics Protocol

1

MIOP Term	Value
analyses	DNA extraction [OBI:0000257]
audience	Scientists
broad-scale environmental context	marine biome ENVO_00000447
creator	Jacoby Baker, https://orcid.org/0000-0002-0673-7535
environmental medium	sea water [ENVO:00002149] | filter paper [OBI:00001511]
geographic location	Monterey Bay [GAZ:00002509]
hasVersion	1
issued	2024-08-22
language	en
license	
local environmental context	oceanic epipelagic zone biome [ENVO:01000033]
materials required	centrifuge [OBI:0400106] | incubator [OBI:0000136]]
maturity level	Demonstrated
methodology category	sample extraction and purification
personnel required	1
project	Marine Biodiversity Observation Network (MBON)
publisher	Monterey Bay Aquarium Research Institute, Chavez Lab
purpose	DNA extraction [OBI:0000257]
skills required	sterile technique | pipetting skills
target	DNA
time required	1320

See https://github.com/BeBOP-OBON/miop/blob/main/model/schema/terms.yaml for list and definitions.

AUTHORS

2

PREPARED BY All authors known to have contributed to the preparation of this protocol, including those who filled in the template.	AFFILIATION	ORCID (visit https://orcid.org/ to re
OSU Technician	Oregon State University	
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RELATED PROTOCOLS

3

PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE / ACCESS DATE
Sterile technique, pipetting skills.	Omega Bio-Tek	2024-01-01

This is a list of other protocols which should be known to users of this protocol. Please include the link to each related protocol.

ACRONYMS AND ABBREVIATIONS

4

	ACRONYM / ABBREVIATION	DEFINITION
	PVDF	polyvinylidene difluoride

GLOSSARY

5

	SPECIALISED TERM	DEFINITION
	Content Cell	Content Cell
	Content Cell	Content Cell

BACKGROUND

6 **Summary**

This protocol is a modified version of the Omega Bio-Tek Mag-Bind Blood & Tissue DNA HDQ 96 Kit used for DNA extractions at Oregon State University's **Center for Quantitative Life Sciences**

7 **Method description and rationale**

This modified protocol is used to extract environmental DNA from filtered seawater samples. It has been applied to 0.22µm PVDF and 0.45µm PVDF.

8 **Spatial coverage and environment(s) of relevance**

This protocol has been used to extract DNA from filtered sea water samples taken from marine coastal stations.

sea water [ENVO:00002149]
http://purl.obolibrary.org/obo/ENVO_00002149

9 **Personnel Required**

1 Technician

10 **Safety**

Identify hazards associated with the procedure and specify protective equipment and safety training required to safely execute the procedure

11 **Training requirements**

Sterile technique, pipetting skills.

12 **Time needed to execute the procedure**

Specify how much time is necessary to execute the procedure.

EQUIPMENT

13

	DESCRIPTION e.g. filter	PRODUCT NAME AND MODEL Provide the official name of the product	MANUFACTURER Provide the name of the manufacturer of the product.	QUANTITY
	Durable equipment			
	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit v8.2	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit v8.2	Omega Bio-Tek	Content Ce

DESCRIPTION e.g. filter	PRODUCT NAME AND MODEL Provide the official name of the product	MANUFACTURER Provide the name of the manufacturer of the product.	QUANTITY
bead beater	TissueLyser II	Qiagen	
Consumable equipment			
homogenization plate	Qiagen collection microtubes	Qiagen	1
5 mm stanless steel bashing beads	Stainless Steel Beads, 5 mm	Qiagen	96
KingFisher tip comb	KingFisher 96 tip comb for deep-well magnets, 10 x 10 pcs/box (for Flex and Presto)		1
2ml deep well plates	KingFisher 96 deep-well plate, sterile (for Duo Prime, Flex and Presto)	KingFisher	
96-well Microplate	e.g., Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/clear	e.g., Bio Rad	
Chemicals			
TL Buffer	TL Buffer	Omega Bio-Tek	
Proteinase K Solution	Proteinase K Solution	Omega Bio-Tek	
Molecular grade Ethanol			
NF H2O	Nuclease Free Water	Invitrogen	
RNase A	RNase A	Omega Bio-Tek	

STANDARD OPERATING PROCEDURE

14 The following protocol is modified from the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit Protocol (https://omegabiotek.com/product/tissue-and-blood-kit-genomic-dna-isolation-mag-bind-hdq-96/?cn-reloaded=1).

Modified portions are **Bolded**.

PREPARATION

15 Before Starting:
• Prepare SPM Buffer, VHB Buffer, and HDQ Binding Buffer according to the "Preparing Reagents" section on Page 4 of the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit Protocol (https://omegabiotek.com/product/tissue-and-blood-kit-genomic-dna-isolation-mag-bind-hdq-96/?cn-reloaded=1).
• Set heat block, incubator, or water bath to *56*°C.

EXTRACTION

16 500
Prepare a mastermix of AL Buffer and Proteinase K Solution only for samples to be extracted according to the table below:

Component	Amount to Prep	Total Amount per 96-well Plate
AL Buffer	**500 µL**	**52.8 mL***
Proteinase K Solution	**40 µL**	**4.2 mL***

* 10% excess volume has been calculated for a 96-well plate.
Important: Only prepare as much AL Buffer/Proteinase K Solution mastermix that will be used within 4 hours of preparation.

17 **Transfer filters into homogenization plate (Qiagen collection microtubes with 5mm stainless steel bead in each tube) with 500 uL TL buffer and 40 uL ProK**

18 **Bead beat for 2x45 seconds at 30 Hz on Qiagen Tissue Lyser II**

19 **Incubate overnight at 56C**

20 Add 5 uL RNase A to each sample

21 **Spin at 4000 x g for 10 minutes**

22 **Transfer 200 uL lysate 2x into two KingFisher Deep Well plates filled with 230 uL AL buffer, pipette mix**

23 **Add 320 uL HDQ binding buffer to both KingFisher Deep Well plates**

24 **Add 20 uL HDQ mag beads to one KingFisher Deep Well plate**

25 Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

25.1 Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

25.2 **Repeat steps 25 & 25.1 with the second KingFisher Deep Well plate that did not include the HDQ mag beads**

26 Remove the plate from the magnetic separation device.

27 Add 600 µL VHB Buffer.
Note: VHB Buffer must be diluted with 100% ethanol prior to use.

28 Vortex for 15 seconds to mix.
Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

29 Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

30 Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

31 Remove the plate from the magnetic separation device.

32 Repeat Steps 27-31 for a second VHB Buffer step.

33 Add 600 µL SPM Buffer.
Note: SPM Buffer must be diluted with 100% ethanol prior to use.

34 Vortex for 15 seconds to mix.

35 Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

36 Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

37 Select one of the following ethanol removal steps:

37.1 A. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water (not provided), leave on magnet for 20-30 seconds, and then aspirate.

Do not leave nuclease-free water on Mag-Bind® Particles HDQ for more than 60 seconds. Continue to Step 40.
OR
B. Leave the plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Bind® Particles HDQ for an additional 10 minutes. Continue to Step 40.

38 Remove the plate from the magnetic separation device.

39 Add **100** µL Elution Buffer to elute DNA from the Mag-Bind® Particles HDQ.
Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

40 Vortex for 5 minutes to mix.
Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

41 Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.

42 Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

QUALITY CONTROL

43 Extracted DNA concentrations were Quantified with ThermoFisher Quant-iT dsDNA HS assay on a BioTek Synergy H1 Hybrid Multi-Mode plate reader

BASIC TROUBLESHOOTING GUIDE

44 Identify known issues associated with the procedure, if any.

Provide troubleshooting guidelines when available.

REFERENCES

45 This protocol is a modified version of the Omega Bio-Tek Mag-Bind Blood & Tissue DNA HDQ 96 Kit (https://omegabiotek.com/product/tissue-and-blood-kit-genomic-dna-isolation-mag-bind-hdq-96/?cn-reloaded=1) used for DNA extractions at Oregon State University's **Center for Quantitative Life Sciences**

APPENDIX A: DATASHEETS

46 Link templates (e.g. preformatted spreadsheets) used to record measurements and report on the quality of the data as well as any documents such as manufacturer specifications, images, etc that support this protocol. Please include a short note describing the document's relevance.

Protocol references

https://omegabiotek.com/product/tissue-and-blood-kit-genomic-dna-isolation-mag-bind-hdq-96/?cn-reloaded=1