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Sucrose lysis buffer V.1

Forked from DNA EXTRACTION Protocol Template

This protocol is a draft, published without a DOI.

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

Draft!

Abstract

This protocol describes the preparation of sucrose lysis buffer to preserve DNA on sterivex filters. The protocol is part of the Hakai Institute's pipeline to analyze microbial and environmental DNA from seawater samples and is implemented as a standard procedure for ongoing sampling programs.

Materials

	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 Provide qua
	Durable equipment			
	Content Cell	Content Cell	Content Cell	Content Cell
	Content Cell	Content Cell	Content Cell	Content Cell
	Consumable equipment			
	Content Cell	Content Cell	Content Cell	Content Cell
	Content Cell	Content Cell	Content Cell	Content Cell
	Chemicals			
	Content Cell	Content Cell	Content Cell	Content Cell
	Content Cell	Content Cell	Content Cell	Content Cell

Before start

Read background information, MIOP and BePOP-OBON information under the "Guidelines" tab.

METHODS

1

> **Note**
>
> Wear gloves.

Equipment:

1. [M] 1 Molarity (M) Tris pH 8 (made previously or purchased as solution)
2. [M] 0.5 Molarity (M) EDTA pH 8 (made previously or purchased as solution)
3. Sucrose
4. MilliQ
5. 500 mL bottle top filtration unit

Final concentrations of chemicals in SLB:
EDTA: [M] 40 Molarity (M)
Tris: [M] 50 Molarity (M)
Sucrose: [M] 0.75 Molarity (M)

2 Calculate how much Sucrose powder you need (using the molecular weight on the bottle, MW or FW) for a [M] 0.75 Molarity (M) solution of 500 mL .

Calculation of Sucrose:

$$\frac{Y\ g}{mol} \times \frac{0.75\ mol}{L} \times 0.5\ L = Z\ grams\ of\ Sucrose\ to\ add\ in\ step\ 2$$

Where Y is the molecular weight of the sucrose (MW or FW) from the bottle.

3 Weigh out and add appropriate amount of Sucrose calculated in step 1 and place in a bottle or beaker.

4 Add 40 mL of [M] 0.5 Molarity (M) EDTA to the beaker.

Calculation of Tris or EDTA:
Use the equation C1V1 = C2V2

Eg for EDTA:
(0.5M)(X mL) = (0.04M)(500 mL)
Solve for X
((0.04M)(500 mL)/(0.5M)) = 40 mL of 0.5M EDTA

5 Add 25 mL of [M] 1 Molarity (M) Tris to the beaker.

Calculation of Tris or EDTA:
Use the equation C1V1 = C2V2

Eg for EDTA:
(0.5M)(X mL) = (0.04M)(500 mL)
Solve for X
((0.04M)(500 mL)/(0.5M)) = 40 mL of 0.5M EDTA

6 Add milliQ water to about the 400 mL line.

7 Add a stir bar and dissolve all powder.

8 Top up water to 500 mL. (no need to pH this one!)

9 Filter-sterilize and label bottle.