

# *E*xtraction of total protein from *Ectocarpus*

1. Freeze the sample in liquid nitrogen directly after harvesting.
2. Grind with a mortar and pestle under liquid nitrogen using some sand.
3. Add 10 ml of lysis buffer per gram of ground tissue and place on ice.
4. Agitate gently for 1 hour on a wheel at 4°C (in a cold room).
5. Centrifuge for 15 minutes at 10,000 g and 4°C.
6. Recover the supernatant and stock at -20°C.

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## SOLUTIONS

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### LYSIS BUFFER

<u>Reagent</u>	<u>Concentration</u>
Glycerophosphate	60 mM
PNPP	15 mM
MOPS	25 mM
Phenylphosphate	1 mM
EGTA	15 mM
DTT	2 mM
MgCl <sub>2</sub>	15 mM
NaVO <sub>4</sub>	1 mM
NaF	1 mM
PVPP	1%
NP40	0.5%

Antiprotease "Complete" (Roche)