



GSe Medium – Modification of G Medium

Saline medium for estuarine and coastal Dinoflagellates

Reference: Blackburn, S. I.; Bolch, C. J. S.; Haskard, K. A., and Hallegraeff, G. M. (2001) Reproductive Compatibility Among Four Global Populations of the Toxic Dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *Phycologia*; 40(1):78-87.

GSe Medium – selenium is added as an important trace element.

Note about Salinity: In G Medium and derivatives the final preparation steps require mixing of nutrients with seawater and dH₂O in a 3:1 or 4:1 ratio. The fully marine seawater (~33-36 practical salinity units) used in ANACC means the resulting media salinity is ~28 psu. In our culturing experience this is an optimal salinity for estuarine and coastal flagellate species, particularly dinoflagellates.

STOCK SOLUTIONS	CONCENTRATION: g L ⁻¹ DEIONISED WATER (dH ₂ O)	VOLUME FOR CONCENTRATED NUTRIENT STOCK
1. KNO ₃	100 g	20 mL
2. K ₂ HPO ₄	34.8 g	10 mL
3. Vitamins	<i>see recipe below</i>	10 mL
4. PII Metal Mix	<i>see recipe below</i>	50 mL
5. Selenium (as selenite) H ₂ SeO ₃	1.29 mg	10 mL
6. Soil Extract	<i>see Soil Extract protocol</i>	

Store all stock solutions in the refrigerator.

Vitamins solution

Add constituents to 100 mL of dH₂O. Remake solution after 3 months.

CONSTITUENT	CONCENTRATION: mg L ⁻¹ DEIONISED WATER (dH ₂ O)	QUANTITY FOR WORKING STOCK
Vitamin B ₁₂	100 mg	2.0 mL
Biotin	100 mg	1.0 mL
Thiamine HCl	<i>add reagent directly to stock</i>	100.0 mg

PII Metal mix

Add the Na₂EDTA to ~750 mL of dH₂O and stir over low heat to dissolve. Add each of the other constituents separately to ~200 mL of dH₂O and fully dissolve between additions. Combine solutions and make up to 1 L. Adjust pH to 7.8 - 8.0 with NaOH.

CONSTITUENT	QUANTITY
Na ₂ EDTA.2H ₂ O	6.0 g
FeCl ₃ .6H ₂ O	290 mg
H ₃ BO ₃	6.85 g
MnCl ₂ .4H ₂ O	860 mg
ZnCl ₂	60.0 mg
CoCl ₂ .6H ₂ O	26.0 mg

GSe Medium Preparation Method

There are 4 components as follows:

1. Seawater

- Autoclave seawater (0.22 µm filtered) in 1000 mL Teflon bottles to sterilise.

2. Deionised Water

- Autoclave dH₂O to sterilise.

3. GSe concentrated nutrients (excluding soil extract)

- Add each stock solution (1 – 5) in the stated quantities and make up to 200 mL with dH₂O.
- Pour into a 250 mL Schott bottle.
- Autoclave at 121°C (15 psi, 20 mins). Alternatively, filter sterilise using a 0.22 µm filter into a sterile 250 mL Schott bottle.

Use 2 mL per 100 mL sterile seawater and dH₂O (3:1 ratio) adding the correct amount of nutrients aseptically.

4. Soil Extract solution

- See Soil Extract protocol for details.

Use Soil Extract at a concentration of 0.5 mL per 100 mL medium.

To prepare GSe Medium (5 L)

In a sterile 5000 mL Schott bottle add aseptically:

- sterile seawater (1) - 4000 mL
- sterile dH₂O (2) - 1000 mL
- sterile GSe concentrated nutrients (3) - 100 mL
- sterile Soil Extract solution (4) - 25 mL

Mix. This medium is now ready to be dispensed aseptically into sterile culture flasks

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