

1723a: Enriched Artificial Seawater Medium f/2 suppl. with Wheat Grains

1. Prepare 1l artificial sea water in milliQ water using:

Artificial Sea Salt (hw Marinemix 21010, Wiegandt GmbH, Sterkenhofweg 13, 47807 Krefeld, Germany), ~ 18 mS/cm = ~ 12 psu at 25°C

12.40 g/l

2. Then add the following components:

NaNO ₃ (75 g/l stock solution)	1.00	ml/l
NaH ₂ PO ₄ x H ₂ O (5 g/l stock solution)	1.00	ml/l
f/2 Trace Metal Mix	1.00	ml

3. Autoclave for 20 min at 121°C.

4. After cooling, add the following filter sterilized (0.2 µm) components to complete the medium:

Vitamin 3 Mix	1.00	ml
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5. The strain is cultivated in 25 cm³ plastic tissue culture flasks with filter screw caps (T25, distributor: TPP, order number: 90026). The T25 flasks are routinely filled with 20 ml of fresh f/2 medium each. Each T25 culture is supplemented with a sterilized wheat grain as carbon source for ambient bacteria from the sampling site.

Wheat Grains (sterilized by autoclaving at 121°C for 20 min)	traces
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f/2 Trace Metal Mix (from medium 1723)

Na ₂ -EDTA x 2 H ₂ O	4.16	g/l
FeCl ₃ x 6 H ₂ O	3.15	g/l
MnCl ₂ x 4 H ₂ O (0.4 g/100 ml stock solution)	45.00	ml/l
ZnSO ₄ x 7 H ₂ O (1.2 g/l stock solution)	18.30	ml/l
CoCl ₂ x 6 H ₂ O (0.6 g/100 ml stock solution)	1.70	ml/l
CuSO ₄ x 5 H ₂ O (1.25 g/100 ml stock solution)	0.80	ml/l
Na ₂ MoO ₄ x 2 H ₂ O (0.27 g/100 ml stock solution)	2.20	ml/l

Vitamin 3 Mix (from medium 1723)

Thiamine HCl (1 g/100 ml stock solution)	1.00	ml
D-Biotin (0.05 g/100 ml stock solution)	0.10	ml
Cyanocobalamin (Vitamin B12; 0.2 g/l stock solution)	0.25	ml