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:
   - NaNO₃ (75 g/l stock solution)  1.00 ml/l
   - NaH₂PO₄ x H₂O (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

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

**f/2 Trace Metal Mix** (from medium 1723)
- Na₂-EDTA x 2 H₂O  4.16 g/l
- FeCl₃ x 6 H₂O  3.15 g/l
- MnCl₂ x 4 H₂O (0.4 g/100 ml stock solution)  45.00 ml/l
- ZnSO₄ x 7 H₂O (1.2 g/l stock solution)  18.30 ml/l
- CoCl₂ x 6 H₂O (0.6 g/100 ml stock solution)  1.70 ml/l
- CuSO₄ x 5 H₂O (1.25 g/100 ml stock solution)  0.80 ml/l
- Na₂ MoO₄ x 2 H₂O (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