



Allen's Cyanidium Medium

Allen 1959, Watanabe et al. 2000

This medium was developed to culture the hot spring acidophilic *Cyanidium* (Allen 1959). The major components presented below are the same as Allen's original recipe; Watanabe *et al.* (2000) vary some major components slightly. However, we include the trace metal solution of Watanabe *et al.* (2000). Allen's original recipe included the same trace elements, at a slightly lower concentration, but it did not include a chelator. Also, Allen added 1×10^{-3} M H_2SO_4 to acidify the medium. We recommend adjusting the pH after all components are added because of the presence of EDTA.

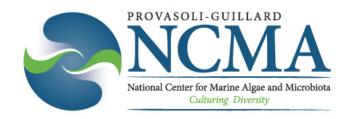
To prepare, begin with 900 mL of dH_20 , individually dissolve the following components and add 100 μ L of the trace metals solution. Adjust to pH 2.5 using 1 N sulfuric acid. Autoclave.

Component	Stock Solution	Quantity	Molar Concentration in
			Final Medium
$(NH_4)_2SO_4$		1.32 g	1.00 x 10 ⁻⁵ M
K ₂ HPO4		0.349 g	2.00 x 10 ⁻⁶ M
MgSO ₄ 7H ₂ O		0.247 g	$1.00 \times 10^{-6} M$
CaCl ₂	55.5 g L ⁻¹ dH ₂ O	1 mL	$5.00 \times 10^{-7} M$
trace metals solution	(see recipe below)	100 μL	

Trace Metals Solution

Watanabe et al. 2000

First, prepare the primary stock solutions. To prepare the final trace metals solution, begin with 900 mL of dH_2O . First, dissolve the Fe-EDTA, then individually add the metal compounds.



Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
Fe-Na-EDTA 3H ₂ 0		0.3016 g	7.16 x 10 ⁻⁸ M
H ₃ BO ₃		0.0289 g	4.67 x 10 ⁻⁸ M
MnCl ₂ 4H ₂ O		0.0179 g	9.04 x 10 ⁻⁹ M
(NH ₄) ₆ Mo ₇ O ₂₄ 4H ₂ O	13.00 g L ⁻¹ dH ₂ O	1 mL	1.05 x 10 ⁻⁹ M
ZnSO ₄ 7H ₂ O	2.20 g L ⁻¹ dH ₂ O	1 mL	7.65 x 10 ⁻¹⁰ M
CuSO ₄ 5H ₂ O	0.79 g L ⁻¹ dH ₂ O	1 mL	3.16 x 10 ⁻¹⁰ M
NH ₄ VO ₃	0.23 g L ⁻¹ dH ₂ O	1 mL	1.97 x 10 ⁻¹⁰ M

Allen, M.B. 1959. Studies with *Cyanidium caldarium*, an anomolously pigmented chlorophyte. *Arch. Mikrobiol.* **32**: 270-7.

Watanabe, M.M., Kawachi, M., Hiroki, M. and Kasai, F. (Eds.) 2000. NIES Collection List of Strains. 6th Ed. NIES, Japan. 159 pp.