

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_2O , individually dissolve the following components and add 100 μL 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
$(\text{NH}_4)_2\text{SO}_4$	---	1.32 g	1.00×10^{-5} M
K_2HPO_4	---	0.349 g	2.00×10^{-6} M
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	---	0.247 g	1.00×10^{-6} M
CaCl_2	$55.5 \text{ g L}^{-1} \text{ dH}_2\text{O}$	1 mL	5.00×10^{-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 O	---	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 anomalously 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.