Culture Collection
CCCryo
of Cryophilic Algae

Fraunhofer
IZI-BB

Spirulina Medium

Aiba, S. & Ogawa, T. (1977): Assessment of growth yield of a blue-green alga, *Spirulina platensis*, in axenic andcontinuous culture. - *J. Gen. Microbiol.* 102(1): 179-182
Andersen, R.A. (ed.) (2005): Algal culturing techniques, 578pp, Elsevier Academic Press, London
modified according to: Schlösser, U.G. (1994): SAG-Sammlung von Algenkulturen at the University of Göttingen. Catalogue of strains. - *Botanica Acta* 107: 111-186

For 1000 mL final culture medium dissolve the following salts in the volume of de-ionized or distilled water as indicated:

Stock Solution SL	Volume	Component	Concentration SL	Conc. Final Medium
SL I	500 mL	$NaHCO_3$	13.61 g	$1.62 \cdot 10^{-1}$ M
		Na_2CO_3	4.03 g	$3.80 \cdot 10^{-2}$ M
		K_2HPO_4	0.50 g	$2.87 \cdot 10^{-3}$ M
		SL I results in a pH of 9.0…9.2		
SL II	500 mL	$NaNO_3$	2.50 g	$2.94 \cdot 10^{-2}$ M
		K_2SO_4	1.00 g	$5.74 \cdot 10^{-3}$ M
		NaCl	1.00 g	$1.71 \cdot 10^{-2}$ M
		$MgSO_4 \cdot 7H_2O$	0.20 g	$8.11 \cdot 10^{-4}$ M
		$CaCl_2 \cdot 2H_2O$	0.04 g	$2.72 \cdot 10^{-4}$ M
		$FeSO_4 \cdot 7H_2O$	0.01 g	$3.60 \cdot 10^{-5}$ M
		Na_2EDTA (Titriplex III)	0.08 g	$2.15 \cdot 10^{-4}$ M
		Micronutient Solution (see SL III and IV below)	5.0 mL	-
		SL II results in a pH of pH = 3.8…4.0		

For liquid medium: Autoclave solutions I and II separately, unite after cooling and add $5 \cdot 10^{-6}$ g L^{-1} vitamin B_{12} in sterile solution, if required (usually vitamins are only required for long-term cultures/stock cultures, however, some algal species require some vitamins). Stock solutions should be stored at 4 °C.
Final pH after combining the autoclaved SL I + SL II = 9.3.

For agarised medium: Prepare liquid medium **from double strength stock solutions I and II**, autoclave distilled water separately and, after cooling, combine 1 part stock I + 1 part stock II + 2 parts distilled water (see below).

For agar slants / petri dishes:

1. Prepare **double strength stock solutions I and II**, i.e. use component concentrations stated in table above and dissolve in 250 mL volume (instead of 500 mL). Autoclave each solution separately.
2. Prepare agar solution in double strength (for 1 L final solution, 1.0 % (agar slants) up to 1.5 % (for agar plates):
   - 500 mL distilled water + 10 up to 15 g Agar Agar
   - heat in microwave to dissolve
   - autoclave
   - let cool to +50…+60 °C
3. Combine 250 mL each of autoclaved solutions I and II and 500 mL of autoclaved agar solution
   - mix and add vitamin B_{12} aseptically
4. Final pH will be 9.3

Preparation of the micronutrient solution (SL III + SL IV)
For 1000 mL final micronutrient solution prepare the following individual stock solutions of each micronutrient in 100 mL of distilled water and add the volumes / amounts indicated to the final volume of distilled water as indicated for SL III and SL IV). Autoclave SL III and SL IV separately to avoid precipitation of salts, let cool and combine both to final Micronutient Solution to obtain the final Spirulina Medium.

Stock Solution SL	Volume	Component	individual SL Concentration	Conc. Final Medium
SL III (total volume = 900 mL)	1 mL	$ZnSO_4 \cdot 7H_2O$	0.1 g · 100 mL^{-1}	$1.74 \cdot 10^{-8}$ M
	2 mL	$MnSO_4 \cdot 4H_2O$* (!)	0.1 g · 100 mL^{-1}	$4.48 \cdot 10^{-8}$ M
	5 mL	H_3BO_3	0.2 g · 100 mL^{-1}	$8.09 \cdot 10^{-7}$ M
	5 mL	$Co(NO_3)_2 \cdot 6H_2O$	0.02 g · 100 mL^{-1}	$1.72 \cdot 10^{-8}$ M
	5 mL	$Na_2MoO_4 \cdot 2H_2O$	0.02 g · 100 mL^{-1}	$2.07 \cdot 10^{-8}$ M
	1 mL	$CuSO_4 \cdot 5H_2O$	0.0005 g · 100 mL^{-1}	$1.00 \cdot 10^{-10}$ M
	0.4 g	Na_2EDTA (Titriplex III)		$1.07 \cdot 10^{-5}$ M
	881 mL	distilled water		
SL IV (total volume = 100 mL)	0.7 g	$FeSO_4 \cdot 7H_2O$		$1.26 \cdot 10^{-5}$ M
	0.4 g	Na_2EDTA (Titriplex III)		$1.07 \cdot 10^{-5}$ M
	100 mL	distilled water		

* at CCCryo 0.1 g $MnSO_4 \cdot 4H_2O$ is replaced by 0.0755 g $MnSO_4 \cdot H_2O$