

ASP12A Recipe for culturing *Aureococcus anophagefferens*

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Abstract

Defined Medium for *Aureococcus anophagefferens*

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Guidelines

Anhydrous Salts

Constituent	Final Conc.	Molar Mass	g/1.0L	g/0.75L	g/0.5L	g/0.25L
NaCl	4.28E-01	58.44	25.01232	18.75924	12.50616	6.25308
KCl	1.10E-02	74.5513	0.8200643	0.615048225	0.410032	0.205016
Na ₂ SO ₄	1.00E-02	142.04	1.4204	1.0653	0.7102	0.3551
NaHCO ₃	2.00E-03	84.007	0.168014	0.1260105	0.084007	0.042004

Hydrous Salts

Constituent	Final Conc.	Molar Mass	g/1.0L	g/0.75L	g/0.5L	g/0.25L
CaCl ₂ ·2H ₂ O	1.25E-02	147.02	1.83775	1.3783125	0.918875	0.459438
MgCl ₂ ·6H ₂ O	4.00E-02	203.3027	8.132108	6.099081	4.066054	2.033027

Macronutrients

Constituent	Final Conc.	Molar Mass	g/1.0L	g/0.75L	g/0.5L	g/0.25L
NaNO ₃	1.47E-03	84.9947	0.124942209	0.093706657	0.062471	0.031236
Ca ₂ -glycerophosphate	2.00E-05	210.14	0.0042028	0.0031521	0.002101	0.001051
K ₂ HPO ₄	4.00E-05	174.2	0.006968	0.005226	0.003484	0.001742

Other

Constituent	Final Conc.	Molar Mass	g/1.0L	g/0.75L	g/0.5L	g/0.25L
Tris Base	4.13E-03	121.14	0.5003082	0.37523115	0.250154	0.125077

Stock Solutions

Solution	Final Conc.	g/L	mL/1.0L	mL/0.75L	mL/0.5L	mL/0.25L
Fe-EDTA sodium Salt	1.79E-06	0.65702	1	0.75	0.5	0.25
TSM - I			1	0.75	0.5	0.25
TSM - II			1	0.75	0.5	0.25
TSM - III			1	0.75	0.5	0.25

S3 Vitamin Solution			1	0.75	0.5	0.25
Trace Metal Solution - TMS - I						
Constituent	Final Conc.	Molar Mass	g/1.0L 1000X Stock			
Na2EDTA·2H2O	8.53E-05	372.24	31.752072			
FeCl3·6H2O	1.79E-06	270.3	0.483837			
H3BO3	2.77E-04	61.83	17.12691			
MnCl2·4H2O	9.10E-06	197.91	1.800981			
ZnSO4·7H2O	1.07E-06	287.56	0.3076892			
NaMoO4·2H2O	5.21E-06	218.9779	1.140874859			
CoCl2·6H2O	2.21E-07	237.93	0.05258253			
Trace Metal Solution - TMS - II						
Constituent	Final Conc.	Molar Mass	g/1.0L 1000X Stock			
KBr	1.25E-04	119.002	14.87525			
SrCl2 · 6H2O	3.42E-05	266.62	9.118404			
RbCl	2.34E-06	120.92	0.2829528			
Trace Metal Solution - TMS - III						
Constituent	Final Conc.	Molar Mass	g/1.0L 1000X Stock			
Na2SeO3	2.00E-08	172.94	0.0034588			
NaF	5.26E-05	41.98871	2.208606146			
NaAsO2	4.00E-08	129.91	0.0051964			
Al2(SO4)3·18H2O	1.11E-06	666.43	0.7397373			
NH4VO3	2.00E-08	116.98	0.0023396			
S3 Vitamin Solution						
Constituent	Final Conc.	Molar Mass	g/1.0L 1000X Stock			
Thiamine HCL	2.96E-07	337.27	0.09983192			
biotin	4.10E-09	244.31	0.001001671			
vitamin B12	7.40E-10	1.36E+03	0.0010064			

Protocol

Step 1.

Prepare Trace Metal Solutions I - III, Fe-EDTA Sodium Salt Solution, and Vitamin Solution. Add constituents to 750 mL MilliQ water, shake to dissolve. Add MilliQ water to 1.0 L. Filter sterilize. Trace Metal Solutions and Fe-EDTA Sodium Salt Solution are stored at 4°C in 15 - 50 mL aliquots, while the Vitamin solution is stored at -20°C in 5 - 10 mL aliquots.

Step 2.

Add anhydrous salts, hydrous salts, macronutrients and Tris Base to 75% desired MilliQ water while stirring to dissolve. Add MilliQ water to final volume, autoclave.

Step 3.

Once cooled, add in 1mL of the Trace Metal Solutions, Fe-EDTA Sodium Salt Solution, and Vitamin Solution per 1L of media made.