

M9 mineral medium

For 1 liter **M9 mineral medium** add to 867 ml sterile water:

100 ml	M9 salt solution (10X)	Na ₂ HPO ₄	33.7 mM
		KH ₂ PO ₄	22.0 mM
		NaCl	8.55 mM
		NH ₄ Cl	9.35 mM
20 ml	20% glucose	glucose	0.4 %
1 ml	1 M MgSO ₄	MgSO ₄	1 mM
0.3 ml	1 M CaCl ₂	CaCl ₂	0.3 mM
1 ml	biotin (1 mg/ml)	biotin	1 µg
1 ml	thiamin (1 mg/ml)	thiamin	1 µg
10 ml	trace elements solution (100X)	trace elements	1X

Stock solutions

M9 salt solution (10X)	Na ₂ HPO ₄ -2H ₂ O	75.2 g/L
	KH ₂ PO ₄	30 g/L
	NaCl	5 g/L
	NH ₄ Cl	5 g/L

Dissolve the salts in 800 ml water and adjust the pH to 7.2 with NaOH. Add water to a final volume of 1 L and autoclave for 15 min at 121°C.

20% Glucose	20% (w/v) glucose	200 g/L
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For 500 ml stock solution add 100 g glucose to 440 ml water. Autoclave for 15 min at 121°C.

1 M MgSO₄	1 M MgSO ₄ -7H ₂ O	24.65 g/100 ml
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For 100 ml stock solution dissolve 24.65 g MgSO₄-7H₂O in 87 ml water. Autoclave for 15 min at 121°C.

1 M CaCl₂	1 M CaCl ₂ -2H ₂ O	14.70 g/100 ml
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For 100 ml stock solution dissolve 14,70 g CaCl₂-2H₂O in 94.5 ml water. Autoclave for 15 min at 121°C.

Biotin (1 mg/ml)	biotin (1mg/ml)	50 mg/50 ml
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For 50 ml stock solution dissolve 50 mg biotin in 45 ml water. Add small aliquots of 1N NaOH until the biotin has dissolved. Add water to a final volume of 50 ml. Sterilize the solution over a 0.22- μ M filter. Prepare 1 ml aliquots and store at -20°C.

Thiamin (1 mg/ml)	thiamin-HCl (1mg/ml)	50 mg/50 ml
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For 50 ml stock solution dissolve 50 mg thiamin-HCl in 45 ml water. Add water to a final volume of 50 ml. Sterilize the solution over a 0.22- μ m filter. Prepare 1 ml aliquots and store at -20°C.

100X trace elements solution	EDTA	5 g /L	13.4 mM
	FeCl ₃ -6H ₂ O	0.83 g/L	3.1 mM
	ZnCl ₂	84 mg/L	0.62 mM
	CuCl ₂ -2H ₂ O	13 mg/L	76 μ M
	CoCl ₂ -2H ₂ O	10 mg/L	42 μ M
	H ₃ BO ₃	10 mg/L	162 μ M
	MnCl ₂ -4H ₂ O	1.6 mg/L	8.1 μ M

Dissolve 5 g EDTA in 800 ml water and adjust the pH to 7.5 with NaOH. Then add the other components in the quantities mentioned below and add water to a final volume of 1 L. Sterilize the solution over a 0.22- μ m filter.

498 mg	FeCl ₃ (anhydrous)	
84 mg	ZnCl ₂	
765 μ l	0.1 M CuCl ₂ -2H ₂ O	1.70 g/100 ml
210 μ l	0.2 M CoCl ₂ -6H ₂ O	4.76 g/100 ml
1.6 ml	0.1 M H ₃ BO ₃	0.62 g/100 ml
8.1 μ l	1 M MnCl ₂ -4H ₂ O	19.8 g/100 ml