

9a: VY/2, DILUTED MEDIUM

Final pH: 6.5

Main sol. 9a

Baker's yeast	2.50	g
CaCl ₂ x 2 H ₂ O	1.36	g
Vitamin B ₁₂	0.50	mg
Agar (Difco)	8.00	g
Distilled water	1000.00	ml
KOH, required for pH adjustment		
<i>Main sol. 1118</i> for suspension of freeze-dried cells from ampoules	1.00	ml

Sterilize vitamin B₁₂ separately by filtration. Prepare and store yeast cells as autoclaved stock suspension (5 g baker's yeast/100 ml distilled water, adjust pH to 6.5 and autoclave). Adjust pH of medium to 7.2 with KOH before, and after autoclaving and cooling to 50°C (use pH-indicator paper). For suspension of freeze-dried cells from ampoules add about 0.5 - 1.0 ml medium 1118 (per liter: casitone 3.0 g; calciumchloride dihydrate 0.7 g; magnesiumsulphate heptahydrate 2.0 g) to the vial with freeze dried material.

Main sol. 1118 (from medium 1118)

Casitone	3.0	g
CaCl ₂ x 2 H ₂ O	0.7	g
MgSO ₄ x 7 H ₂ O	2.0	g
<i>Trace element solution SL-4</i>	1.0	ml
Distilled water	1000.0	ml

After autoclaving and cooling, add 0.5 mg /l vitamin B₁₂ from a filter sterilized stock solution. pH should be 7.0 -7.2.

SL107: Trace element solution SL-4 (from medium 1118)

EDTA	0.500	g
FeSO ₄ x 7 H ₂ O	0.200	g
ZnSO ₄ x 7 H ₂ O	0.010	g
MnCl ₂ x 4 H ₂ O	0.003	g
H ₃ BO ₃	0.030	g
CoCl ₂ x 6 H ₂ O	0.020	g
CuCl ₂ x 2 H ₂ O	0.001	g
NiCl ₂ x 6 H ₂ O	0.002	g
Na ₂ MoO ₄ x 2 H ₂ O	0.003	g
Distilled water	1000.000	ml