

9a: VY/2, REDUCED MEDIUM

Final pH: 7.2

Final volume: 1000 ml

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

1. 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).
2. Adjust pH of medium to 7.2 with KOH before as well as after autoclaving and cooling to 50°C (use pH-indicator paper).
3. For suspension of freeze-dried cells from ampoules add about 0.5 - 1.0 ml medium MD1 (per liter: casiton, 3.0 g; CaCl₂ x 2 H₂O, 0.7 g; MgSO₄ x 7 H₂O, 2.0 g) to the vial with freeze dried material.