

**9a. VY/2, REDUCED MEDIUM**

Baker's yeast	2.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.36	g
Vitamin B <sub>12</sub>	0.50	mg
Agar (Difco)	8.00	g
Distilled water	1000.00	ml

Sterilize vitamin B<sub>12</sub> 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 MD1 (per liter: casiton 3.0 g; calciumchloride dihydrate 0.7 g; magnesiumsulphate heptahydrate 2.0 g) to the vial with freeze dried material.