

## 9: VY/2 AGAR

Final pH: 7.2

Final volume: 1000 ml

Baker's yeast	5.00	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)	15.00	g
Distilled water	1000.00	ml

1. 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).

2. Cultures of myxobacteria delivered freeze-dried: Please see our video tutorial and follow the special instructions: 'Reactivation of Myxobacteria' given with the strain entry of our catalogue. For suspending the freeze-dried cells from ampoules, add about 0.5 - 1.0 ml medium MD1 (per liter: casiton 3.0 g; CaCl<sub>2</sub> x 2 H<sub>2</sub>O, 0.7 g; Mg<sub>2</sub>SO<sub>4</sub> x 7 H<sub>2</sub>O, 2.0 g) to the vial with freeze dried material.

3. Cultures of myxobacteria delivered as active cultures (growing on agar plates): Always use the rim of the swarm as inoculum for fresh media. If the swarms are creamy, transfer high amounts of cell mass to several spots on fresh VY/2 agar medium. If the swarm adheres to the agar or grows within the agar, cut small agar cubes from the rim of the swarm and place onto a fresh agar plate using an appropriate tool such as a lancet. Make sure that the pieces of swarm colonies grown on the agar are transferred to the agar plate. Attempt to place the inoculum in such a way that the swarms are in contact with the fresh agar plate.

4. Incubate for up to 3 weeks (in particular Sorangium and Nannocystis strains) at the temperature given for the strain, taking measures against desiccation. If there is no growth after ten days, carefully split up the agar-culture-cubes and squeeze the material to the agar plate and reincubate.