

## Reactivation of freeze-dried myxobacteria

Myxobacteria are freeze-dried either as small cubic cuts from swarms grown on agar medium or as cells and myxospores suspended in a protecting agent.

In both cases, please follow the instructions "[Opening of ampoules](#)" given on the yellow flyer accompanying the DSMZ cultures up to point 6 (opening of the inner vial), that is:

- Wear protective glasses when opening ampoules!
- Heat the tip of the ampoule in a flame.
- Place two or three drops of water onto the hot tip to crack the glass.
- Carefully strike off the glass tip with an appropriate tool (e.g. forceps).
- Remove the insulation material with forceps and take out the inner vial.
- Lift the cotton plug using a forceps, remove it, keep it under sterile conditions and flame the top of the inner vial.

Continue by adding about 0.5 - 1.0 ml medium MD1 (modified DSM medium 1118, see below) to the vial and let the agar cubes rehydrate for 30 min at room temperature.

The small agar cubes are transferred from the vial directly onto an agar plate of DSM medium 9 (VY/2-medium) using an appropriate tool such as an inoculation loop or a lancet. Make sure that the cultures (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.

If the ampoule contains the suspension place 3-5 drops on an agar plate, medium 9, thus using up the suspension completely. Do not re-distribute the drops on the plate, rather incubate the plate with the drops in place.

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 re-incubate.

### Modified Medium MD1

Casitone (pancreatic digest of casein)	3.0	g
Calciumchloride dihydrate	0.7	g
Magnesiumsulphate heptahydrate	2.0	g
Distilled water	1000.0	ml

### VY/2 Agar

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

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).

### **Notes:**

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If you have any questions or comments to this page, please contact the expert on Gram-negative bacteria <https://www.dsmz.de/collection/collection-experts>.