

### **YPGS(Yeast extract Peptone Glucose Saccharose)**

Yeast extract	1.0 g/l
Peptone	10.0 g/l
Glucose	5.0 g/l
Sucrose	5.0 g/l
NaCl	3.0 g/l
	pH 7.0

medium for growth and investigation Liquid medium for bacteria other than root nodulating bacteria, primarily used for reviving freeze-dried cultures.

### **SMA (Standard Methods Agar)**

Standard Methods Agar 'Nissui'	23.5 g/l
Agar	3.0 g/l

medium for most bacteria.

Though Standard Methods Agar 'Nissui' contains agar, we add 3.0g/l more agar to it in order to stiffen it and inoculate easier.

### **medium for *Bradyrhizobium***

Yeast extract	0.4 g/l
Mannitol	10.0 g/l
K <sub>2</sub> HPO <sub>4</sub>	0.5 g/l
MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.2 g/l
NaCl	0.1 g/l
Agar	18.0 g/l
	pH 6.8

for root nodulating bacteria, *Bradyrhizobium*. For *Rhizobium*, Add 3.0 g of CaCO<sub>3</sub> to this medium.

### **TTC(2,3,5-Triphenyl Tetrazolium Chloride)**

Peptone	10.0 g/l
Casein hydrolysate	1.0 g/l
Glucose	5.0 g/l
1% solution of TTC	1ml g/l
Agar	15.0 g/l

medium for *Ralstonia solanacearum*. TTC solution (filtered through a 0.2μ filter) should be added at 50-60°C after autoclaving a mixture of other components.

### **MRS (de Man-Rogosa-Sharpe)**

MRS medium 'OXOID'	52.0 g/l
Agar	18.0 g/l

for lactic acid bacteria.

### **King B**

King B Agar 'Eiken' 38.0 g/l

Glycerin 10.0 g/l

King B medium is a confirmation medium for the identification of fluorescein-producing *Pseudomonas*.

### **King's B medium**

10 g proteose peptone #2 (DIFCO)

1.5 g anhydrous  $K_2HPO_4$

15 g glycerol

5 mL  $MgSO_4$  (1 M; sterile)

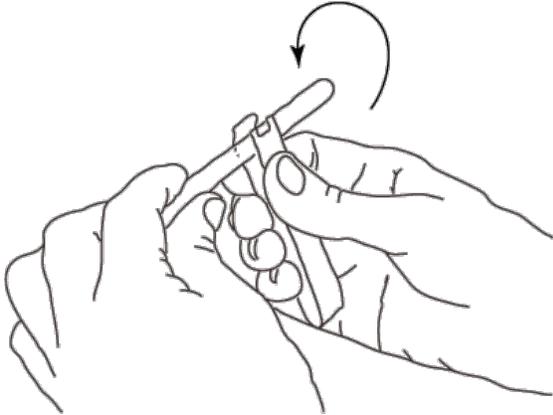
Antibiotics (as needed)

Add  $H_2O$  to first three ingredients to bring volume to 1 L. Adjust the pH to 7.0 with HCl. Autoclave and then add 5 mL of sterile 1 M  $MgSO_4$ . If  $MgSO_4$  is added before autoclaving, the medium becomes cloudy.

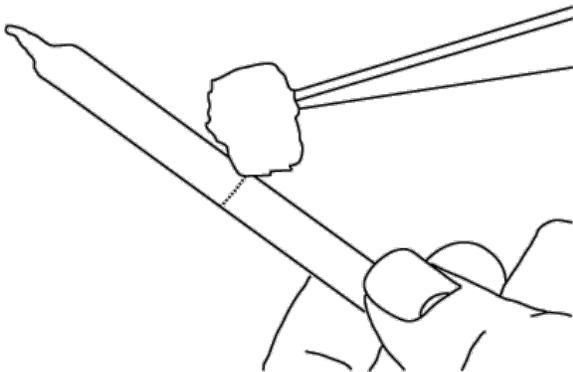
## Instruction for Reviving Freeze-dried Cultures

Manipulate following procedures as carefully and aseptically as possible, using a clean bench. Moreover, store the ampoules in the refrigerator when they are not opened immediately.

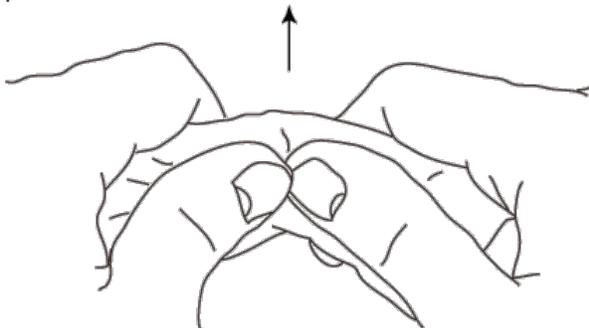
1. Prepare liquid media, solid culture media and sterilized Pasteur pipettes. Letters on ampoules may be vanished by the sterilizing alcohol. Affix to the ampoules paper labels with the information necessary for identification.
2. Score the central part of the ampoules with an ampoule cutter.



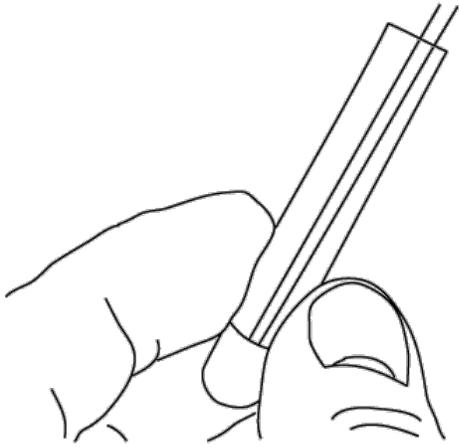
3. Sterilize the surface of the ampoule with absorbent cotton wetted with 70% ethanol.



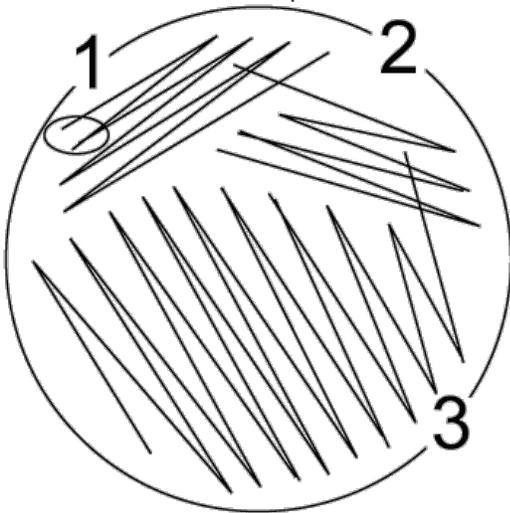
4. Cover the ampoules with sterilized gauze. Then break the ampoules carefully at the scored part.



5. Add 0.1ml of liquid media or sterilized water to the ampoule and suspend the dried samples.



6. Aspirate the dissolved samples and drip it on a plate. Streak the suspension as illustrated below. Flame the loop after the 1st and 2nd streak.



7. Incubate the plate. It may take a double-period of general culture, since these cells are weak because of freeze-drying.