

STORAGE OF PHYTOPHTHORA IN LIQUID NITROGEN

Safety notice: always wear eye and hand protection when handling liquid nitrogen and cryovials!

A. FREEZING

Materials needed

Sterile 15% v/v DMSO in water

2 ml cryovials (Nunc 3-68632 or equivalent)

#4 Cork borer

Rack

Procedure

1. For standard storage, prepare 5 vials per isolate (label with isolate number and month/year, i.e. 4.57 1 11/94).
2. Flame sterilize #4 cork borer using 70% ethanol.
3. Using the cork borer cut out 5 plugs 0.5 to 1.5 cm from the growing edge of colony.
4. Use a rod (or a smaller cork borer) to push out the 5 plugs into a vial.
- 5- Add 1 ml of 15% DMSO to each vial. This should leave at least 10% head space in the vial to allow expansion.
6. Gently screw on the caps (do not overtighten)
7. Place in controlled freezing unit and activate (will cool at about 1°/min; program is on next page).
8. When done cooling, transfer to storage unit.
9. RECORD THE POSITION OF THE SAMPLES ON THE DATA SHEET!

B. THAWING

1. Remove vial from nitrogen storage and place in a 37°C water bath for 2-4 minutes.
2. Using forceps, remove plugs from vial and distribute on 2 rye agar plates.
3. Incubate at 18°C. Growth should be seen within one week.
4. RECORD THE REMOVAL OF THE SAMPLES ON THE DATA SHEET

Program for cryofreezing

This is the program for the Planer Kryo10 freezer unit. It contains two programs, linked together.

Program 1

RAMP 1	+20 C to 0 in 20 min.	RATE -1.00/min, TEMP +0.0
RAMP 2	Hold for 10 min @ 0 C	RATE -00.0/min, TEMP n/a, TIME 0.10
RAMP 3	0 to -10 C in 6.3 min	RATE -1.59/min, TEMP -10.0
RAMP 4	immediately to -40 C	RATE -50.00/min, TEMP -40.0
RAMP 5	-40 to -37 C in 0.3 min	RATE +3.75/min, TEMP -37.0
RAMP 6	Hold for 1.5 min @ -37 C	RATE -00.0/min, TEMP n/a, TIME 0.01 (or 0.02)
RAMP 7	-37 to -19 in 3.4 min	RATE +5.29/min, TEMP -19.0
RAMP 8	-19 to -44 in 16 min	RATE -01.56/min, TEMP -44.0

Program 2 (linked to program 1)

RAMP 1	-44 to -120 in 10 min	RATE -07.6/min, TEMP -120
RAMP 2	Hold for 20 min @ -120 C	RATE -00.0/min, TEMP n/a, TIME 0.20

Near the end of this time, transfer the rack to liquid nitrogen unit. (don't let warm up!)