Bodo saltans culture protocol Version 2

Fatma Gomaa,ZhuHong Li,Roberto Docampo,Peter Girguis,Virginia Edgcomb

Abstract

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Guidelines

Culture Maintenance:

1. Prepare the bacterized *Bodo saltans* medium as described above.
2. Inoculate a T25 tissue culture flask (50ml) containing 20 to 25 ml of fresh medium with 1 to 2 ml from Bodo culture that is at or near peak density
3. Incubate horizontally at 18 to 22°C (room temperature can work fine) with cap screwed on not very tightly. 4. Subculture every 7 to 10 days.
4. I usually subculture 3 to 4 flasks every week

Before start

Medium recipe:

ATCC medium: 802 Sonneborn's Paramecium medium Solution 1 Rye grass Cerophyll:

Cerophyll*.....2.5 g

Distilled water.....1.0 L

Add cerophyll to distilled water and boil for 5 minutes.

Add 100 ml distilled water to compensate for evaporation.

Filter through Whatman #1 filter paper and add 0.5 g Na2HPO4.

Autoclave for 15 minutes at 121C.

1. saltans food (*K. pneumoniae*, or *E. coli*). I used only the *K. pneumoniae* so far

Agar Medium for *Klebsiella pneumoniae* ATCC-BAA-1705:

Agar.....20.0 g

Yeast extract.....4.0 g

Glucose.....0.16 g

Distilled water.....800.0 ml

Dispense in 5 ml amounts. Autoclave for 25 minutes at 121C. Slant. Bacterium, grown on solution 2, is added to solution 1 (Just add very little, few colonies) and incubated at 30C for 24 hours prior to inoculation with Bodo saltans.

- Cerophyl powder that works best for the *saltans* is the powder from Pines.

Protocol

Cryopreservation: Harvest and Preservation

Step 1.

Harvest cells from a culture that is at peak density by centrifugation at 800 x g for 5 min.

DURATION

00:05:00 : Centrifugation

Cryopreservation: Harvest and Preservation

Step 2.

Adjust the concentration of cells to 2 x $10^6$to 10^7 /mL in fresh medium (Important step, even for transfection).

Cryopreservation: Harvest and Preservation

Step 3.

Prepare a 20% (v/v) solution of sterile DMSO in fresh Bodo medium.

REAGENTS

20% (v/v) solution of sterile DMSO by Contributed by users

Cryopreservation: Harvest and Preservation

Step 4.

Add 2.0 mL of DMSO to an ice cold tube

AMOUNT
2 ml : DMSO

Cryopreservation: Harvest and Preservation

Step 5.

Place the tube on ice and allow the DMSO to solidify (5 min) and then add 8.0 mL of ice cold medium.

AMOUNT
8 ml : ice cold medium
DURATION
00:05:00 :

Cryopreservation: Harvest and Preservation

Step 6.

Invert several times to dissolve the DMSO.

Cryopreservation: Harvest and Preservation

Step 7.

Allow to warm to room temperature

Cryopreservation: Harvest and Preservation

Step 8.

Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10^6 to $10^7$and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no longer than 30 min.

DURATION
00:15:00 :

Cryopreservation: Harvest and Preservation

Step 9.

Dispense in 0.5 mL aliquots into 1.0 mL to 2.0 mL sterile plastic screwcapped cryules (special plastic vials for cryopreservation).

Cryopreservation: Harvest and Preservation

Step 10.

Place the vials in a controlled rate freezing unit. From room temperature cool at 1°C/min to - 40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at
1°C/min through the heat offusion. At -40°C plunge into liquid nitrogen. Alternatively, place the vials i n Nalgene 1°C freezing
apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately 1°C/min.)

Cryopreservation: Harvest and Preservation

Step 11.

To establish a culture from the frozen state place an ampule in a water bath set at +35°C. Immerse th e ampule to a level just above the surface of the frozen material. Do not agitate the ampule.

Cryopreservation: Harvest and Preservation

Step 12.
Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate a T25 tissue culture flask containing 10 mL of Bodo medium bacterized with Klebsiella pneumoniae subsp. pneumoniae (ATCC® 700831).

Cryopreservation: Harvest and Preservation

Step 13.
Incubate horizontally with the cap screwed on tightly at 22°C

TEMPERATURE
22 °C :