Bodo saltans culture protocol

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

Abstract

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.

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

Cryopreservation:

Harvest and Preservation:

1. Harvest cells from a culture that is at peak density by centrifugation at 800 x g for 5 min. 2. Adjust the concentration of cells to 2 x $10^6$to 10^7 /mL in fresh medium (Important step, even for transfection).
2. Prepare a 20% (v/v) solution of sterile DMSO in fresh Bodo medium.
3. Add 2.0 mL of DMSO to an ice cold tube
4. Place the tube on ice and allow the DMSO to solidify (~5 min) and then add 8.0 mL of ice c old medium.
5. Invert several times to dissolve the DMSO.
6. Allow to warm to room temperature
7. 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 sto ck solution

before the freezing process is begun should be no less than 15 min and no longer than 30 min.

5. Dispense in 0.5 mL aliquots into 1.0 mL to 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. 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 in 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.)
7. To establish a culture from the frozen state place an ampule in a water bath set at +35°C. Immerse the ampule to a level just above the surface of the frozen material. Do not agitate the ampule.
8. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate a T-25 tissue culture flask containing 10 mL of Bodo medium bacterized with Klebsiella pneumoniae subsp. pneumoniae (ATCC® 700831).
9. Incubate horizontally with the cap screwed on tightly at 22°C

Citation: Fatma Gomaa,ZhuHong Li,Roberto Docampo,Peter Girguis,Virginia Edgcomb Bodo saltans culture protocol. **protocols.io**

dx.doi.org/10.17504/protocols.io.sh2eb8e
Published: 09 Aug 2018

Protocol