BIOMEDICAL RESEARCH INSTITUTE
NIH NIAID Schistosomiasis Resource Center

9410 Key West Avenue
Rockville, MD 20850
Phone 1-301-329-0412
Fax 1-301-881-7640
www.afbr-bri.org

Biomphalaria glabrata embryonic cell (Bge) culture protocol

Authors: Wannaporn Ittiprasert, PhD
André Miller

Materials

Bge Medium 1 liter:

- Schneider's Drosophila Medium 220 mL
- lactalbumin hydrolysate 4.5 g
- Galactose 1.3g
- MilliQ H2O up to 900mL
- Gentamycin (10 mg/mL stock) 2.0 mL
- Phenol Red (0.5% solution) 1.62mL
  Adjust pH to 7.0
  Sterile filter at least > 0.22 micron

Grow cells at 26°C in complete Bge medium (7-10% heat-inactivated FBS in Bge medium)
Keep the cells growing at a high density. This cell line likes to form cell balls on top of a monolayer. Split the cells when the cell balls get very dense or about once a week. Split the cells only 1:2, keeping the cell culture dense.

To split/passage Bge cells: remove the attached cells by using a cell scraper (not trypsin) and transfer the suspension into a 15 ml tube. Resuspend cells in 1 mL complete Bge medium and use 0.1-0.2mL of cell suspension to re-seed into new 50 mL tissue culture flask with 5 mL complete Bge medium.

To freeze Bge cells: remove the attached cells by using a cell scraper and transfer the cell suspension into a 15 mL tube. Centrifuge at 700 rpm for 5 min. Re-suspend cells in 0.5-1 mL of complete Bge medium, then count cells by cell counter. Dilute the cells to a final concentration at 10^9-10^{10} cells/mL in room temperature-freezing medium [9 FBS: 1 DMSO (v/v)]. Aliquot 0.8-1.5 mL into freezing vials then transfer into freezing container (pre-cool down in refrigerator; 0-4°C) as soon as possible. Keep freezing container in -70°C for overnight, then move the vial to liquid nitrogen storage.

To revive frozen cells from liquid nitrogen: thaw cells in 35-37°C water bath until ~80% is thawed, then add 1-2 ml of Bge medium at room temperature immediately. Transfer the cell suspension into a 15 mL tube and centrifuge at 700 rpm for 5 min, room temperature (RT). Resuspend the cell pellet with at least 7 mL of Bge medium and centrifuge as above. Resuspend the cells with 5 mL complete Bge medium, and then transfer the cells to 50 mL-tissue culture flask. Let the cells rest in culture at 26°C for a week (DO NOT CHANGE MEDIUM) to let cells adapt and start dividing before changing medium.

Comments

Bge cells are sent in a 50mL culture flask filled with Bge medium. We ship the flask overgrown to insure cell culture health during transit. Upon receipt, allow cells to settle for 1-2hr. Decant all but 6mL of medium. The cells can then be split into as many as a dozen flasks. Bge cells are not easily retrieved from frozen vials, therefore it is best to keep a culture growing at all times.

*For more information contact André Miller at **amiller@afbr-bri.org***