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Biomphalaria glabrata embryonic cell (Bge) culture protocol

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Materials

Bge Medium 1 liter:

- Schneiders'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 FCS 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 in to 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 not easily retrieved from frozen vials, therefore it is best to keep a culture growing at all times.

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