Live *Biomphalaria glabrata* Embryonic (Bge) Cell Line

Catalog No. NR-40248

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Contributor and Manufacturer:

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Product Description:

The *Biomphalaria glabrata* embryonic (Bge) cell line was derived from embryos of the snail *Biomphalaria glabrata,* M-line, by Eder Hansen in 1976.[1] The original karyotype of this cell line was identical to that of the intact snail (n = 18), however, the Bge cell line now exhibits extensive aneuploidy.[2,3] Bge cells are contact-inhibited upon reaching confluency.[2]

Material Provided:

Live Bge cells, that are approximately 70% confluent, are provided in freshly prepared Bge medium in 50 mL tissue culture flasks. Please see Appendix I for manufacturer recommended protocols.

Packaging/Storage:

Flask(s) are shipped overnight at room temperature. After receiving the cells maintain the flask at approximately 26°C overnight to allow the cells to settle after transportation. The next day, remove most of the transport media, leaving approximately 4 to 5 mL of the old medium. Allow the cells to grow and then refer to the manufacturer's cell culture methods for further passaging (Appendix I).

Citation:

Acknowledgment for publications should read "The following reagent was provided by the NIAID Schistosomiasis Resource Center for distribution through BEI Resources, NIAID, NIH: Live *Biomphalaria glabrata* Embryonic (Bge) Cell Line, NR-40248."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Hansen, E. L. "A Cell Line from Embryos of *Biomphalaria glabrata* (Pulmonata): Establishment and Characteristics." Invertebrate Tissue Culture: Research Applications Ed. K. Maramorosch. USA: Academic Press Inc. 1976, 77-97.
2. Dr. Matty Knight, Personal communication
3. Odoemelam E., et al. "Revised Karyotyping and Gene Mapping of the *Biomphalaria glabrata* Embryonic (Bge) Cell Line." Int. J. Parasitol. 39 (2009): 675-681. PubMed: 19133265.

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APPENDIX I

Bge Cell Culture

Bge cells are not easily retrieved from frozen vials, therefore, it is best to keep a culture growing at all times.

Bge cells should be grown at 26°C in complete Bge medium [10% heat-inactivated fetal bovine serum (FBS) in Bge medium].

Keep the cells growing at a high density. Bge cells may form cell balls on top of a monolayer, which is acceptable, however, when the cell balls get very dense they should be split (about once per week). Split the cells only 1:2, it takes a long time for them to recover from a thin plating, so it is important to keep them dense. Change the medium about once per week. The cells won't come off easily but can be removed with a cell scraper.

Complete Bge medium (1 L)

Schneiders's Drosophilia Medium	220 mL
Lactalbumin hydrolysate	4.5 g
Galactose	1.3 g
Gentamycin (10 mg/mL stock)	2.0 mL
Phenol Red (0.5% solution)	1.62 mL solution or 0.81 mg powder
NOTE: too much phenol red will kill the cells	
MilliQ water	up to 900 mL

Adjust pH to 7.0
Sterile filter
Add heat inactivated FBS to a final of either 10% or 5% v/v before use. Note: If only 5% FBS is added, additional MilliQ water will need to be added to bring the volume up to 1 L.

Heat-inactivation of FBS (Manufacturer uses FBS from Hyclone)
Preheat a water bath to 56°C and place tubes of FBS into it. Measure the temperature of the beaker in which the tubes are and wait until it has reached 56°C. This is especially important if you start with frozen tubes. Once the FBS has reached 56°C then incubate at this temperature for 30 min. Store the heat-inactivated FBS at -20°C. Note: *Bge cells are very sensitive to different lots of FBS therefore it is important to test new lots.*

Splitting Bge cells:
Frequently splitting Bge cells shortens their longevity in tissue culture. Passage cells 2 to 3 weeks after they have been split. In between passaging, the medium can be changed once. To split the cells, remove the attached cells with a cell scraper and transfer the cell suspension to a 15 mL tube. Resuspend the cells in 1 mL of complete Bge medium and use 0.1 mL to 0.2 mL of the cell suspension to reseed a new 50 mL tissue culture flask with 3 mL to 5 mL of complete Bge medium. Bge cells can also be maintained in complete medium with only 5% FBS.

Freezing Bge cells:
Remove the attached cells with a cell scraper and transfer the cell suspension to a 15 mL tube. Centrifuge at 700 rpm for 5 min. Resuspend the cells in 0.5 mL to 1 mL complete Bge medium, then count the cells with a cell counter. Dilute the cells to a final concentration of 10^9 to 10^{10} cells/mL in room temperature freezing medium [9 FBS:1 DMSO (v/v)]. Aliquot 0.8 mL to 1.5 mL into freezing vials then transfer into a pre-cooled freezing container (0°C to 4°C) as soon as possible. Store freezing container at -70°C overnight then transfer vials to liquid nitrogen tank for long term storage [cryogenic temperature (-100°C or colder), preferably in the vapor phase of a liquid nitrogen freezer]. Storage at -70°C will result in loss of viability.

Reviving frozen cells from liquid nitrogen:
Thaw cells in a 35°C to 37°C water bath until the cells are approximately 80% liquid (a small piece of ice should still remain in the tube) then immediately add 1 mL to 2 mL complete Bge medium at room temperature. Transfer the cell suspension into a 15 mL tube and centrifuge at 700 rpm for 5 min at room temperature. Resuspend the cell pellet with 5 mL of complete Bge medium and transfer the cells to a 50 mL tissue culture flask (non-vented Falcon catalog number 3014, blue cap or Falcon catalog number 3081 black cap). Tighten the cap of the flask and incubate the culture at 26°C (without CO_2) for a week. **DO NOT CHANGE THE MEDIA**, it is very important to let the cells adapt and start dividing before changing the media.

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