Biomphalaria glabrata Embryonic (Bge) Cell Line

Catalog No. NR-21959

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For research use only. Not for human use.

Contributor and Manufacturer:

Matty Knight, Principal Investigator, Biomedical Research Institute, Rockville, MD (NIH-NIAID Contract HHSN272201000005I)

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:

Each vial of lot 05202011 contains approximately 1.5 mL of cell culture suspension frozen in fetal calf serum (90%) and DMSO (10%). Each vial of lots 090111 and 01292011 contains approximately 1 mL of cell culture suspension frozen in fetal calf serum (90%) and DMSO (10%). Bge cells are not easily retrieved from frozen vials, please see Appendix I for manufacturer recommended protocols.

Packaging/Storage:

This product was packaged in plastic vials. It should be stored at 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. To insure the highest level of viability, the vial should be thawed and the culture initiated as soon as possible upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product after thawing. For transfer between freezers and shipping, the cells may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred.

Safety Precautions:

When handling frozen vials it is highly recommended that protective gloves, lab coat and full face mask be worn. Even brief exposure to the ultra-cold temperature can cause tissue damage from frostbite. Also, some vials may slowly fill with liquid nitrogen if they have been immersed during cryogenic storage. When thawing, the liquid nitrogen may rapidly expand as it changes to gas, breaking the vial or cap with explosive force, sending debris flying with enough velocity to cause injury. Store and use in areas with adequate ventilation.

Citation:

Acknowledgment for publications should read "The following reagent was provided by the NIAID Schistosomiasis Resource Center for distribution through BEI Resources, NIAID, NIH: *Biomphalaria glabrata* Embryonic (Bge) Cell Line, NR-21959."

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 http://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

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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 very 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.*

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.

Splitting Bge cells:
Frequently splitting Bge cells shortens their longevity in tissue culture, and therefore, routinely 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.

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