

4a-3B, *Anopheles gambiae* Cell Line

Catalog No. MRA-919

For research use only. Not for human use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

The *Anopheles gambiae* cell line 4a-3B was established from neonate larvae of the 4a r/r strain.^{1,2}

Material Provided:

Each vial of MRA-919 contains approximately 0.5 mL of 4a-3B cells in Schneider's Insect medium supplemented with 10% fetal bovine serum (FBS) and 10% dimethylsulfoxide (DMSO). Please see Appendix I for media preparation. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells per vial, is shown on individual certificates of analysis for each product lot.

Packaging/Storage:

This product was packaged aseptically, in screw-capped plastic cryovials. 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. Please read the following recommendations prior to reconstituting this material.

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.

Growth Conditions:

Prior to thawing the cells, prepare culture medium according to Appendix I. Thaw 1 vial in a 25°C water bath and transfer

the contents into a 25-mL vented cell culture flask with 9 mL of culture medium. Keep the flask tightly capped in a 25°C incubator (no CO₂ required). Change the media at 12 to 16 hours post seeding. Feed the cells at least every 48 hours, harvest at 80%-90% confluency and reseed at a 1:3 to 1:5 ratio.

Sub-culture procedure: When cells near confluency, detach cells by vigorous shaking, mechanical disruption or gentle cell scraping. Collect and gently aspirate several times with a pipette to disrupt clumped cells prior to cell counting as required and passage to new flasks.

Note: Trypsin or trypsin-like enzyme substitute may be used to fully disperse adherent cells but is not recommended on a continuous basis for MRA-919.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: 4a-3B, *Anopheles gambiae* Cell Line, MRA-919, contributed by George K. Christophides."

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/bmb15/index.htm.

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References:

1. Christophides, G. K., Personal Communication.
2. Müller, H.-M., et al. "A Hemocyte-Like Cell Line Established from the Malaria Vector *Anopheles gambiae* Expresses Six Prophenoloxidase Genes." J. Biol. Chem. 274 (1999): 11727-11735. PubMed: 10206988.
3. Dimopoulos, G., et al. "*Anopheles gambiae* Pilot Gene Discovery Project: Identification of Mosquito Innate Immunity Genes from Expressed Sequence Tags Generated from Immune-Competent Cell Lines." Proc. Natl. Acad. Sci. USA 97 (2000): 6619-6624. PubMed: 10841561.
4. Dimopoulos, G., et al. "Genome Expression Analysis of *Anopheles gambiae*: Responses to Injury, Bacterial Challenge, and Malaria Infection." Proc. Natl. Acad. Sci. USA 99 (2002): 8814-8819. PubMed: 12077297.

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APPENDIX I: MEDIA PREPARATION

Culture Medium

Schneider's Insect medium
10% FBS (qualified for insect cell culture or heat-inactivated)

Facultative:

100 U/mL Penicillin
100 U/mL Streptomycin

Freezing Medium

Schneider's Insect medium
10% FBS
10% DMSO