Product Information Sheet for MRA-917

4a-2, *Anopheles gambiae* Cell Line

Catalog No. MRA-917

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
The *Anopheles gambiae* cell line 4a-2 was established from neonate larvae of the 4a r/r strain.[1]

Material Provided:
Each vial of MRA-917 contains approximately 0.5 mL of 4a-2 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/vial, is shown on individual certificates of analysis for each product lot.

Packaging/Storage:
This product was packaged aseptically in cryovials. It should be stored at -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 ensure 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-cm vented cell culture flask with 9 mL of culture medium. Keep the flask tightly capped in a 27°C to 28°C incubator (no CO_2 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 1:2 to 1:4 ratio.

Subcultivation 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-917.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: 4a-2, *Anopheles gambiae* Cell Line, MRA-917, 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 (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

Disclaimers:
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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. "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.
4. 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.
5. Catteruccia, F., et al. "Toward *Anopheles* Transformation: *Minos* Element Activity in Anopheline Cells and Embryos." Proc. Natl. Acad. Sci. USA 97 (2000): 2157-2162. PubMed: 10681436.

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 µg/mL Streptomycin

Freezing Medium
Schneider's Insect medium
10% FBS
10% DMSO

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