

Hybridoma 6D12 Anti-*Aedes aegypti* Salivary Glands

Catalog No. MRA-249

For research use only. Not for human use.

Contributor:

Kenneth D. Vernick, Professor, Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, New York, USA

Manufacturer:

BEI Resources

Product Description:

The murine hybridoma cell line, 6D12, was generated by the fusion of SP2/0 mouse myeloma cells with splenocytes from BALB/c mice immunized with salivary gland membranes of *Aedes aegypti* (*Ae. aegypti*), strain Black-Eye Liverpool mosquitoes.^{1,2} The monoclonal antibody produced binds preferentially to the distal lateral lobes of female and male *Ae. aegypti* salivary glands.¹ The 6D12 antibody does not cross-react with salivary glands of *Anopheles gambiae* and *Culex pipiens* mosquitoes.^{1,2}

Material Provided:

Each vial contains approximately 0.5 mL of hybridoma cells in cell culture medium supplemented with 10% dimethylsulfoxide (DMSO) at a concentration of 10⁶ cells per mL. Please see Appendix I for media preparation. Sufficient cells are provided to initiate at least one new culture.

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 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.

Functional Activity:

Hybridoma 6D12 produces monoclonal antibody of the IgG1 subclass, which is specific for female and male *Ae. aegypti* salivary glands, and is reported to function in immunoblot and immunofluorescence assays.^{1,2}

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.

Subcultivation Procedure:

Prior to thawing the hybridoma cells, prepare cell culture medium according to Appendix I. Thaw one vial in a 37°C water bath and transfer the contents into a 25-cm cell culture flask with 10 mL of cell culture medium. Keep the flask loosely capped in a 37°C incubator with 5% CO₂. Change media at 12-16 hours post-seeding. Feed cells at least every 48 hours and split cells when 70% confluent.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Hybridoma 6D12 Anti-*Aedes aegypti* Salivary Glands, MRA-249, contributed by Kenneth D. Vernick."

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. Barreau, C., et al. "Identification of Surface Molecules on Salivary Glands of the Mosquito, *Aedes aegypti*, by a Panel of Monoclonal Antibodies." Insect Biochem. Mol. Biol. 29 (1999): 515-526. PubMed: 10406090.
2. Vernick, K. D., Personal Communication.

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APPENDIX I: MEDIA PREPARATION

Cell Culture Medium

Advanced RPMI 1640 medium (Gibco™ 12633; 1x)

Supplemented with:

- Fetal Bovine Serum (FBS, hybridoma-tested; 10%)
- L-glutamine (4 mM)
- Gentamicin (optional; 50 µg per mL)

Freezing Medium

Cell culture medium (as above)
10% DMSO

Freeze cells at 10⁷ per mL