Microglial Cell Line Derived from MAL/MyD88 Double Knockout Mice

Catalog No. NR-9904

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Contributor:

Dr. Douglas Golenbock, Professor, Infectious Diseases and Immunology, The University of Massachusetts Medical School, Worcester, Massachusetts

Manufacturer:

BEI Resources

Product Description:

The murine microglial cell line, NR-9904, was derived using brain tissue from MAL (MyD88 adaptor-like)/MyD88 (myeloid differentiation primary response protein 88) double knockout mice. The microglial cells were immortalized by infection with the ecotropic transforming replication-deficient retrovirus J2 using techniques described in the literature.[1-6] Characterization based on immunofluorescence, stimulation assays and flow cytometry demonstrated that the immortalized cell line retains its microglial-specific morphological, functional and surface expression properties.

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in cell growth medium (90%) and DMSO (10%) cryopreservative. 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.

Thawing and Growth:

Prior to thawing the murine microglial cells, prepare growth medium (GM) for use. Murine microglial cells are grown in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen™ 11960-044) modified to contain 10% irradiated fetal bovine serum (FBS; Lonza 14-471F), 2 mM L-glutamine (Invitrogen™ 25030-081), 1 mM sodium pyruvate (Invitrogen™ 11360-070) and 10 µg/mL ciprofloxacin. This GM is formulated for use with a 5% CO_2 in air atmosphere.

Rapidly thaw the vial of murine microglial cells in a 37°C water bath with gentle agitation. To reduce the risk of contamination, keep the cap and O-ring of the vial out of the water and repeatedly check the cap for tightness during thawing. Remove from the water bath immediately when thawed. Dry the vial with a sterile wiper, decontaminate using a wiper soaked with 70% isopropyl alcohol, and let the vial air dry. Aseptically open the vial, remove the vial contents and add to 4 mL of pre-warmed (37°C for 15 to 30 minutes) GM in a centrifuge tube. Centrifuge the cell suspension at 125 to 200 X g for 8 to 10 minutes at 18 to 25°C. Discard the supernatant and resuspend the cell pellet in 10 mL of pre-warmed GM. Transfer the cell suspension into a 75 cm^2 tissue culture flask. Incubate the new culture at 37°C and 5% CO_2. The cells will take 5 to 7 days to attach to the flask. Do not replace the GM during this time. After the cells have attached, replace the GM with fresh GM every 2 to 3 days until the cell sheet is approximately 80% confluent.

Sub-culture procedure. Aseptically remove the GM and discard. Briefly rinse the cell layer with 4 to 15 mL of Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS; Invitrogen™ 14190-144) to remove all traces of serum. Discard the PBS. Add 2 to 8 mL of 0.05% trypsin-EDTA (Invitrogen™ 25300-062) to the culture flask and incubate the flask at 37°C until cell layer is dispersed (usually within 3 minutes but no longer than 15 minutes). *Note: To avoid clumping, do not agitate the cells by hitting or shaking the flask.* Add an equal volume of GM and aspirate cells by gently pipetting. Perform a cell count and add appropriate aliquots of the cell suspension to new culture vessels at a sub-cultivation ratio of 1:3 to 1:4. Adjust the volume of GM to 15 to 20 mL for a 75 cm^2 flask. Incubate cultures at 37°C and 5% CO_2. Replace the GM with fresh GM every 2 to 3 days and incubate until the cell sheet is approximately 80% confluent. Note: If the media pH becomes too acidic resulting in the detachment of the cells from the flask surface, centrifuge the detached cells at 125 to 200 X g for 8 to 10

minutes at 18 to 25°C. Discard the supernatant and resuspend the cell pellet in 10 mL of pre-warmed GM. Transfer the cell suspension into a 75 cm^2 flask. Allow 5 to 7 days for the cells to re-attach. After the cells have re-attached, replace the GM with fresh GM every 2 to 3 days until the cell sheet is approximately 80% confluent.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Microglial Cell Line Derived from MAL/MyD88 Double Knockout Mice, NR-9904."

Biosafety Level: 2

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:

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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898