

**Microglial Cell Line Derived from TRAM Knockout Mice**

**Catalog No. NR-9903**

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**Product Description:** The murine microglial cell line, NR-9903, was derived using brain tissue from TRAM (TRIF-related adaptor molecule; TRIF: toll-interleukin-1 receptor domain-containing adaptor-inducing interferon-β) knockout mice. The microglial cells were immortalized by infection with the ecotropic transforming replication-deficient retrovirus J2 using techniques described in the literature. 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.

**Lot: 58381524**

**Manufacturing Date: 04NOV2008**

TEST	SPECIFICATIONS	RESULTS
<b>Growth Properties</b>	Adherent	Adherent
<b>Morphology by Immunofluorescence<sup>1</sup></b>	Microglial	Microglial
<b>Surface Marker Expression by Flow Cytometry Using Specific Antibodies<sup>1</sup></b> CD11b F4/80 MHCII GFAP (astrocyte marker)	Characteristic of microglial cells Positive Positive Positive Negative	Characteristic of microglial cells Positive Positive Positive Negative
<b>Multiplex PCR Amplification of Cytochrome C Oxidase I (COI) Gene</b>	Murine origin No evidence of another species	Murine origin No evidence of another species
<b>Total Cell Count</b>	> 1.0 X 10 <sup>6</sup> cells/vial	4.2 X 10 <sup>6</sup> cells/vial
<b>Post-Freeze Viability</b>	≥ 50%	67%
<b>Sterility (BacT/ALERT<sup>®</sup> 3D Microbial Detection System)</b> 14-day incubation of NR-9903: i NST culture bottle, 32°C, anaerobic i AST culture bottle, 32°C, aerobic	No growth No growth	No growth No growth
<b>Mycoplasma Contamination</b> Hoechst DNA stain Agar and broth culture (14-day incubation at 37°C) DNA Detection by PCR of Test Article nucleic acid	None detected None detected None detected	None detected None detected None detected

<sup>1</sup>Performed on immortalized cell line prior to growth of this distribution lot.

**Date:** 06 MAR 2014

**Signature:** *Dorothy C. Young*

**Title:** Technical Manager, BEI Authentication or designee

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