

## **Certificate of Analysis for NR-19978**

## Microglial Cell Line Derived from TLR1 Knockout Mice

Catalog No. NR-19978

This reagent is the property of the U.S. Government.

**Product Description:** This murine microglial cell line was derived using brain tissue from TLR1 knockout mice. The microglial cells were immortalized by infection with the ecotropic transforming replication-deficient retrovirus J2 using techniques described in the literature.

Lot: 60523124 Manufacturing Date: 03MAY2012

TEST	SPECIFICATIONS	RESULTS
Growth Properties	Adherent	Adherent
PCR Amplification of Extracted DNA (Figure 1) TLR1 wild type primers TLR1 knockout primers	No amplicon Expected amplicon	No amplicon Expected amplicon
Stimulation of TNF- $\alpha$	Report results	See Figure 2
Multiplex PCR Amplification of Cytochrome C Oxidase I (COI) Gene	Murine origin No evidence of another species	Murine origin No evidence of another species
Total Cell Count	> 1.0 x 10 <sup>6</sup> cells/vial	5.1 x 10 <sup>6</sup> cells/vial
Post-Freeze Viability	≥ 50%	90.1%
Sterility (21-day incubation) Harpo's HTYE broth <sup>1</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth	No growth
Mycoplasma Contamination  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>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 12 FEB 2014 Signature

Title: Technical Manager, BEI Authentication or designee

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BEI Resources www.beiresources.org E-mail: <a href="mailto:contact@beiresources.org">contact@beiresources.org</a>
Tel: 800-359-7370

Fax: 703-365-2898



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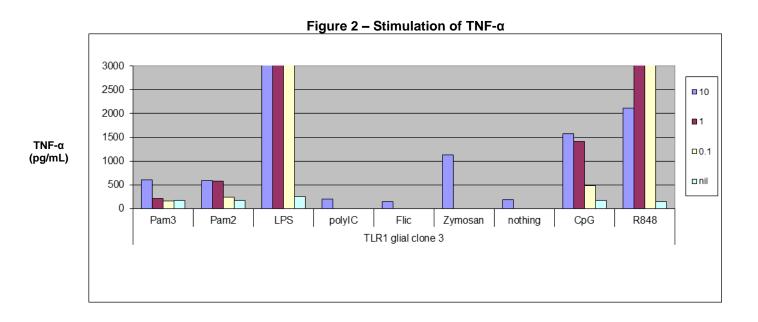
Figure 1 - PCR Amplification



Lane 1: Wild type microglial cells

Lane 2: TLR1 knockout microglial cells (NR-19978)

Lane 3: Negative control (H<sub>2</sub>O)



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