

## **Certificate of Analysis for NR-19976**

## Macrophage Cell Line Derived from TLR2/TLR9 Double Knockout Mice

## Catalog No. NR-19976

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

**Product Description:** This murine macrophage cell line was derived using primary bone marrow cells from TLR2/9 double knockout mice. The macrophage cells were immortalized by infection with the ecotropic transforming replication-deficient retrovirus J2 using techniques described in the literature.

Lot: 63379483 Manufacturing Date: 11MAY2015

TEST	SPECIFICATIONS	RESULTS
Growth Properties	Adherent	Adherent
PCR Amplification of Extracted DNA (Figure 1) <sup>1</sup>	N. P.	N. P.
TLR2 wild type primers TLR2 knockout primers	No amplicon Expected amplicon	No amplicon Expected amplicon
Stimulation of TNF-α <sup>1</sup>	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 per vial	5.1 x 10 <sup>6</sup> cells per vial
Post-Freeze Viability	≥ 50%	87.8%
Sterility (21-day incubation) Harpo's HTYE broth <sup>2</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

Depositor QC is NOT lot specific; same data as NR-19976 lot 60523122

Date: 18 AUG 2015 Signa

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<sup>&</sup>lt;sup>2</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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Figure 1 - PCR Amplification

TLR2 Wild Type Primers

1 2 3

1 2 3

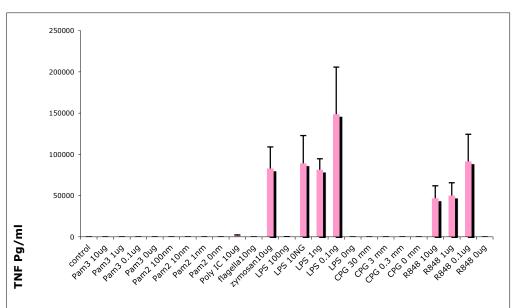
1 2 3

Lane 1: Wild type macrophages

Lane 2: TLR2/9 knockout macrophages (NR-19976)

Lane 3: Negative control (H<sub>2</sub>0)

Figure 2 – Stimulation of TNF-α



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