

# 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:** 60523122

**Manufacturing Date:** 08FEB2012

TEST	SPECIFICATIONS	RESULTS
<b>Growth Properties</b>	Adherent	Adherent
<b>PCR Amplification of Extracted DNA (Figure 1)</b> TLR2 wild type primers TLR2 knockout primers	No amplicon Expected amplicon	No amplicon Expected amplicon
<b>Stimulation of TNF-<math>\alpha</math></b>	Report results	See Figure 2
<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>	$> 1.0 \times 10^6$ cells/vial	$7.3 \times 10^6$ cells/vial
<b>Post-Freeze Viability</b>	$\geq 50\%$	89.0%
<b>Sterility (21-day incubation)</b> 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 No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth 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>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Date:** 10 APR 2014

**Signature:**

*Dorothy C. Young*

**Title:**

Technical Manager, BEI Authentication or designee

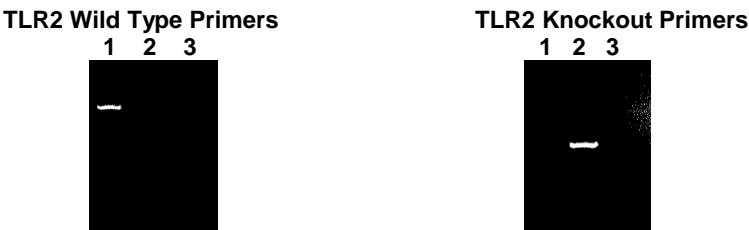
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Figure 1 – PCR Amplification



Lane 1: Wild type macrophages  
Lane 2: TLR2/9 knockout macrophages (NR-19976)  
Lane 3: Negative control (H<sub>2</sub>O)

Figure 2 – Stimulation of TNF-α

