

**Macrophage Cell Line Derived from TLR4 Knockout Mice**

**Catalog No. NR-9458**

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**Product Description:** The murine macrophage cell line, NR-9458, was derived using primary bone marrow cells from TLR4 (toll-like receptor 4) knockout mice. The primary bone marrow cells were immortalized by infection with the ecotropic transforming replication-deficient retrovirus J2 using techniques described in the literature.

**Lot: 70018417**

**Manufacturing Date: 04SEP2018**

TEST	SPECIFICATIONS	RESULTS
<b>Growth Properties</b>	Adherent	Adherent
<b>Morphology by Immunofluorescence<sup>1</sup></b>	Macrophage	Macrophage
<b>Confirmation of Knockout Phenotype by Indirect Fluorescent Antibody Assay</b> TLR4 <sup>2</sup> TLR9 <sup>3</sup>	No fluorescence observed Fluorescence observed	No fluorescence observed Fluorescence observed
<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 × 10 <sup>6</sup> cells per vial	6.3 × 10 <sup>6</sup> cells per vial
<b>Post-Freeze Viability</b>	≥ 50%	78.0%
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>4</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
<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.

<sup>2</sup>Using TLR4 Antibody (Calbiochem®, AP1179)

<sup>3</sup>Using TLR9 Antibody (Novus Biological, NBP1-76680)

<sup>4</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/

Heather Couch

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