

Certificate of Analysis for NR-19974

Macrophage Cell Line Derived from TLR3 Knockout Mice

Catalog No. NR-19974

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 TLR3 knockout mice. The macrophage cells were immortalized by infection with the ecotropic transforming replication-deficient retrovirus J2 using techniques described in the literature.

Lot: 60243377 Manufacturing Date: 03MAY2012

TEST	SPECIFICATIONS	RESULTS
Growth Properties	Adherent	Adherent
PCR Amplification of Extracted DNA (Figure 1) TLR3 wild type primers TLR3 knockout primers	No amplicon Expected amplicon	No amplicon Expected amplicon
Stimulation of RANTES	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 ⁶ cells/vial	3.7 x 10 ⁶ cells/vial
Post-Freeze Viability	≥ 50%	73.6%
Sterility (21-day incubation) Harpo's HTYE broth ¹ , 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 ₂	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

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

Date: 13 FEB 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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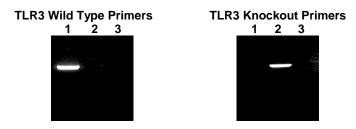
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Figure 1 - PCR Amplification

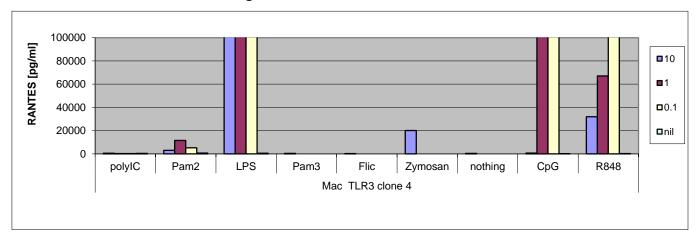


Lane 1: Wild type macrophages

Lane 2: TLR3 knockout macrophages (NR-19974)

Lane 3: Negative control (H₂O)

Figure 2 - Stimulation of RANTES



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