

Certificate of Analysis for NR-19975

Macrophage Cell Line Derived from TLR2/TLR4 Double Knockout Mice

Catalog No. NR-19975

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/TLR4 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: 60523121 Manufacturing Date: 27FEB2012

TEST	SPECIFICATIONS	RESULTS
Growth Properties	Adherent	Adherent
PCR Amplification of Extracted DNA (Figure 1)		
TLR2 wild type primers	No amplicon	No amplicon
TLR2 knockout primers	Expected amplicon	Expected amplicon
TLR4 wild type primers	No amplicon	No amplicon
TLR4 knockout primers	Expected amplicon	Expected amplicon
Stimulation of TNF-α	Report results	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 × 10 ⁶ cells per vial	4.7 × 10 ⁶ cells per vial
Post-Freeze Viability	≥ 50%	96.0%
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ¹	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO ₂	No growth	No growth
Mycoplasma Contamination		
Hoechst DNA stain	None detected	None detected
110001101211110101111		1
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected

¹Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798

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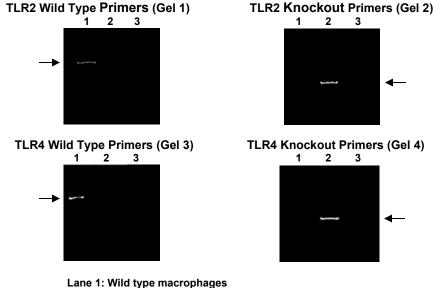
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Figure 1: PCR Amplification

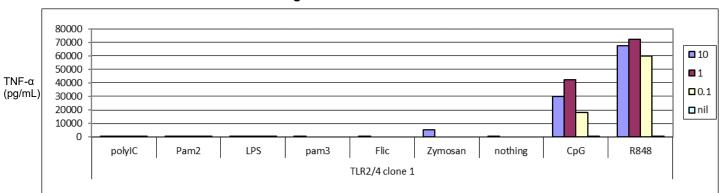


Lane 1: Wild type macrophages

Lane 2: TLR2/TLR4 double knockout macrophages (NR-19975)

Lane 3: Negative control (H₂O)

Figure 2: Stimulation of TNF-α



/Heather Couch/ Heather Couch

15 NOV 2019

Program Manager or designee, ATCC Federal Solutions

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