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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-15633

Macrophage Cell Line Derived from MyD88 Knockout Mice

Catalog No. NR-15633

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 MyD88 knockout mice. The macrophage cells were immortalized by infection with the ecotropic transforming replication-deficient retrovirus J2 using techniques described in the literature.

Lot: 60523117 **Manufacturing Date: 21MAR2012**

TEST	SPECIFICATIONS	RESULTS
Growth Properties	Adherent	Adherent
PCR Amplification of Extracted DNA (Figure 1) MyD88 wild type primers MyD88 knockout primers	 No amplicon Expected amplicon	 No amplicon Expected amplicon
Stimulation of TNF-α	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	3.2 x 10^6 cells/vial
Post-Freeze Viability	≥ 50%	92.7%
Sterility (21-day incubation) Harpo's HTYE broth[2], 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 No growth No growth No growth No growth No growth	 No growth No growth No growth No growth No growth 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

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

Date: 18 FEB 2014 **Signature:** Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

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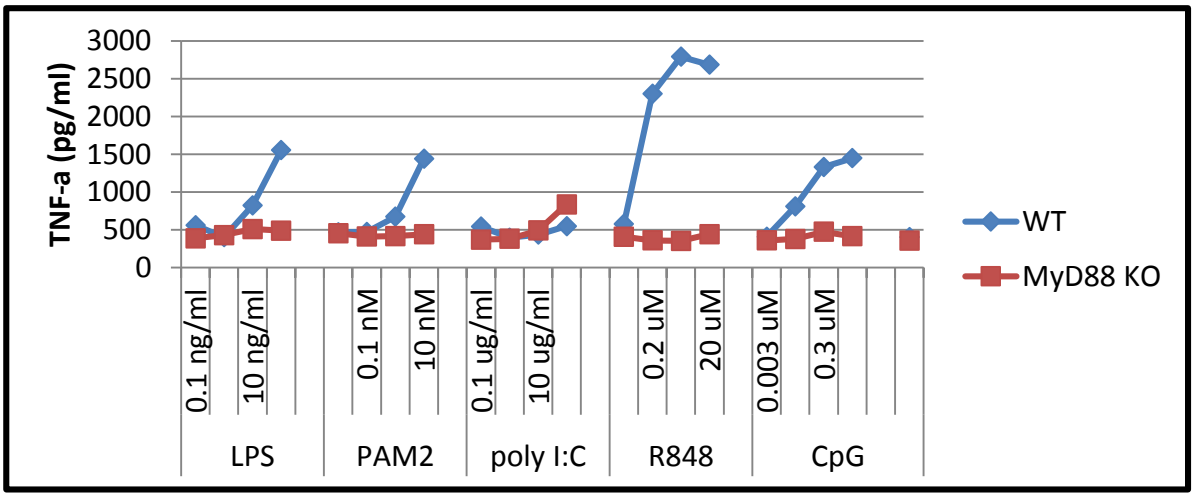
Figure 1 – PCR Amplification

Lane 1: Wild type macrophages
Lane 2: Knockout control
Lane 3: MyD88 knockout macrophages (NR-15633)
Lane 4: Negative control (H_2O)

Figure 2 – Stimulation of TNF-α



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