SUPPORTING INFECTIOUS DISEASE RESEARCH

Macrophage Cell Line Derived from TRIF/MyD88 Double Knockout Mice

Catalog No. NR-15632

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 TRIF/MyD88 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: 60243376

Manufacturing Date: 24FEB2012

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
Stimulation of RANTES	Report results	See Figure 3
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	3.9 x 10 ⁶ cells/vial
Post-Freeze Viability	≥ 50%	60.2%
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 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 ¹ Atlas, Ronald M. <u>Handbook of Microbiological Media</u> . 3rd ed. Ed. La	None detected None detected None detected	None detected None detected None detected

Date: 13 FEB 2014

Signature: Dorothy C. Young

Title:

Technical Manager, BEI Authentication or designee

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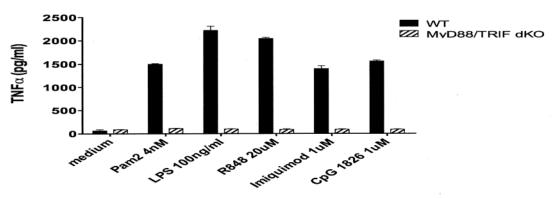
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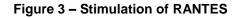
Certificate of Analysis for NR-15632

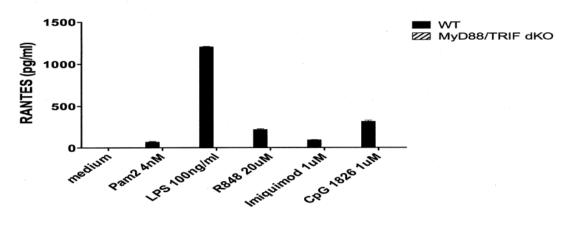
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MyD88 Wild Type Primers 1 2 3 4 1 2 3 4 Lane 1: Wild type macrophages Lane 2: Knockout control Lane 3: TRIF/MyD88 double knockout macrophages (NR-15632) Lane 4: Negative control (H₂O) Figure 2 – Stimulation of TNF-α

Figure 1 – PCR Amplification







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