

Certificate of Analysis for NR-9456

Macrophage Cell Line Derived from Wild Type Mice

Catalog No. NR-9456

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Product Description: The murine macrophage cell line, NR-9456, was derived using primary bone marrow cells from wild type mice. The primary bone marrow cells were immortalized by infection with the ecotropic transforming replication-deficient retrovirus J2 using techniques described in the literature. Characterization based on immunofluorescence, stimulation assays and flow cytometry demonstrated that the immortalized cell line retains its macrophage-specific morphological, functional and surface expression properties.

Lot: 62112678 Manufacturing Date: 30DEC2013

TEST	SPECIFICATIONS	RESULTS
1-01	SPECIFICATIONS	
Growth Properties	Adherent	Adherent
Morphology by Immunofluorescence ¹	Macrophage	Macrophage
Surface Marker Expression by Flow Cytometry Using Specific Antibodies CD11b F4/80 CD45 CD80 CD86 CD14 MHC CD11c (dendritic cell marker) B220 (lymphocyte marker)	Characteristic of bone marrow- derived macrophages Positive Positive Positive Positive Positive Positive Positive Negative Negative	Characteristic of bone marrow- derived macrophages CD11b+++ F4/80++ CD45+ CD80+ CD86+ CD14+ MHC++ CD11c- B220-
Multiplex PCR Amplification of Cytochrome C Oxidase I (COI) Gene	Murine origin No evidence of another species	Murine origin No evidence of another species
Cell Count	> 1.0 × 10 ⁶ cells/vial	7.9 × 10 ⁶ cells/vial
Post-Freeze Viability	≥ 50%	84.6%
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

¹Performed on immortalized cell line prior to growth of this distribution lot

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Date: 08 APR 2014 Signa

Signature: Downg C. Young

Title: Technical Manager, BEI Authentication or designee

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