Certificate of Analysis for NR-791

Ectromelia Virus, Recombinant expressing Mouse Interleukin-4

Catalog No. NR-791

Product Description: Cell lysate and supernatant from African green monkey kidney (BS-C-1) cells[1] infected with interleukin-4 (IL-4) expressing ectromelia virus (ECTV).

Lot[2]: 7578064 **Manufacturing Date: 02NOV2006**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in BS-C-1 Cells[1]	Report results	Cell rounding and detachment
Sequencing of Recombinant-Specific Region (~ 570 bp)	Murine IL-4 gene	Murine IL-4 gene
Murine IL-4 ELISA[3] of Infected Cell Lysate and Supernatant	Reactive	Reactive
Titer by $TCID_{50}$ Assay[4,5] in BS-C-1 Cells[1]	Report results	8.9×10^7 $TCID_{50}$/mL
Functional Activity by RT-PCR Amplification IL-4-specific region ECTV-specific region	 ~ 450 bp amplicon ~ 680 bp amplicon	 ~ 450 bp amplicon ~ 680 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth[6], 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep 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 Agar and broth culture DNA Detection by PCR of Test Article nucleic acid	 None detected None detected	 None detected None detected

[1]BS-C-1 cells: ATCC® CCL-26™ (Lot: 3816047).
[2]Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370) supplemented with 2% irradiated fetal bovine serum (Cambrex® 14-471F), 2 mM L-glutamine (Invitrogen™ 25030), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 4 days at 37°C and 5% CO_2.
[3]Biosource Mouse IL-4 Immunoassay Kit, MS (Invitrogen™ KMC0041).[4]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture.
[4]The $TCID_{50}$ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[5]9 days at 37°C and 5% CO_2.
[6]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 13 AUG 2007 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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