

Certificate of Analysis for NR-2330

Japanese Encephalitis Virus, G 8924

Catalog No. NR-2330

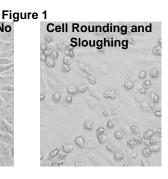
Product Description: Cell lysate and supernatant from African green monkey kidney (Vero) cells¹ infected with Japanese encephalitis virus (JEV), G 8924.

Lot²: 58248360 Manufacturing Date: 04AUG2008

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Report results	Cell rounding and sloughing (Figure 1)
Identification by Indirect Fluorescent Antibody Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of JEV Specific Sequence (~ 960 bp)	Consistent with JEV	Consistent with JEV
Titer by TCID ₅₀ Assay ^{4,5} in Vero Cells ¹	Report results	8.9 X 10 ⁶ TCID ₅₀ /mL
RT-PCR Assay of Extracted RNA Using JEV Specific Primers	~ 1145 bp amplicon	~ 1145 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
Mycoplasma Contamination 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

¹Vero cells: ATCC[®] CCL-81™

Negative Control: No Infectivity



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²Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% irradiated fetal bovine serum (Lonza® 14-471F), 2 mM L-glutamine (Invitrogen™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 6 days at 37°C and 5% CO₂

³Using monoclonal antibody reactive with JEV (Millipore MAB8743)

⁴The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

⁵7 days at 37°C and 5% CO₂

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798



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Date: 14 OCT 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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