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BIODEFENSE & EMERGING INFECTIONS RESEARCH RESOURCES REPOSITORY

Certificate of Analysis for NR-10178

Tamiami Virus, W-10777

Catalog No. NR-10178

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Product Description: Cell lysate and supernatant from African green monkey kidney [VERO C1008 (E6)] cells[1] infected with Tamiami virus (TAMV), W-10777.

Lot[2,3]: 58443731 **Manufacturing Date: 22APR2009**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in VERO C1008 (E6) Cells Using RT-PCR[1,4]	Report results	Infectious; no cytopathic effect
Sequencing of Tamiami Virus S Segment Gene	Consistent with TAMV	Consistent with TAMV
Titer by $TCID_{50}$ Assay in VERO C1008 (E6) Cells Using RT-PCR[1,4]	Report results	8.9×10^5 $TCID_{50}$/mL
Functional Activity by RT-PCR Assay	~ 301 bp amplicon	~ 301 bp amplicon
Sterility (BacT/ALERT® 3D Microbial Detection System) 14-day incubation of NR-10178: i NST culture bottle, 32°C, anaerobic i AST culture bottle, 32°C, aerobic	 No growth No growth	 No growth No growth
Fungal Sterility (21-day incubation) Harpo's HTYE broth[5], 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C and 26°C, aerobic	 No growth No growth No growth	 No growth 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

[1]VERO C1008 (E6) cells: ATCC® CRL-1586™; also available as BEI Resources NR-596

[2]The inoculum for this lot was BEI Resources NR-10178 (Lot 58232458), which was determined by PCR to be contaminated with *Mycoplasma arginini*. Upon three passages in the presence of 0.5 µg/mL mycoplasma removal agent (MP 30-500-44), PCR tests showed the live virus to be clean and free of mycoplasma contamination. Source virus for NR-10178 (Lot 58232458) was deposited by Professor Charles H. Calisher of the Colorado State University, Fort Collins, Colorado.

[3]Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex® 14-471F), 2 mM L-glutamine (Invitrogen™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 14 days at 37°C and 5% CO_2

[4]RNA used for RT-PCR was extracted from infected VERO C1008 (E6) cell lysate and supernatant after 13 days incubation at 37°C and 5% CO_2.

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

Date: 28 FEB 2011 **Signature:** Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

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