b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-9536

Paraná Virus, 12056

Catalog No. NR-9536

This reagent is the tangible property of the U.S. Government.

Product Description: Clarified supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero E6) infected with Paraná virus (PARV), 12056

Lot[1]: 57956741 **Manufacturing Date: 2007**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells[2] Using RT-PCR[3]	Report results	Infectious, no cytopathic effect
Sequencing of Species-Specific Region (345 nucleotides)	Consistent with PARV	99% identity with PARV (GenBank: AF485261)
Titer by $TCID_{50}$ Assay[4] in Vero E6 Cells[1] Using RT-PCR[3]	Report results	8.9×10^6 $TCID_{50}$ per mL
Functional Activity by RT-PCR Assay	~ 350 bp amplicon	~ 350 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[5], 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA Detection by PCR of Test Article nucleic acid	None detected	None detected

[1]Grown and deposited by Charles H. Calisher, Ph.D., Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA
[2]Vero E6 cells; ATCC® CRL-1586™
[3]RNA used for RT-PCR was extracted from infected cell lysate and supernatant after 15 days incubation at 37°C and 5% CO_2.
[4]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. 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]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004. p. 798.

Date: 29 MAY 2015 **Signature:** Michael R. Gyrla

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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898