

Certificate of Analysis for NR-12237

Whitewater Arroyo Virus, AV 9310135

Catalog No. NR-12237

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 Whitewater Arroyo virus (WWAV), AV 9310135

Lot¹: 58454279 Manufacturing Date: 2009

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 (368 nucleotides)	Consistent with WWAV, AV 9310135	99% identity with WWAV, AV 9310135 (GenBank: AF228063)
Titer by TCID ₅₀ Assay ⁴ in Vero E6 Cells ² Using RT-PCR ³	Report results	1.6 × 10 ⁴ TCID ₅₀ per mL
Functional Activity by RT-PCR Assay	~ 380 bp amplicon	~ 380 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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

¹Grown 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

Date: 29 MAY 2014 Signature: Michael Q. Gyptu

Title: Technical Manager, BEI Authentication or designee

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²Vero E6 cells; ATCC[®] CRL-1586™

³RNA used for RT-PCR was extracted from infected cell lysate and supernatant after 14 days incubation at 37°C and 5% CO₂.

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.

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