Certificate of Analysis for NR-10403

Rickettsia rhipicephali, Strain CWPP

Catalog No. NR-10403

Product Description: Cell lysate and supernatant from African green monkey kidney (Vero) cells[1] infected with *Rickettsia rhipicephali*, strain CWPP.

Lot[2]: 58365929 **Manufacturing Date: 06OCT2008**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells[1]	Report results	Small cell rounding
Identification by Sequencing of Citrate Synthase Gene (~ 1020 bp)	*Rickettsia rhipicephali*	*Rickettsia rhipicephali*[3]
Titer by $TCID_{50}$ Assay[4,5] in Vero Cells[1]	Report results	2.8 X 10^5 $TCID_{50}$/mL
PCR Amplification of Extracted DNA	~ 1154 bp amplicon	~ 1154 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[6], 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
Brucella 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 (30-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]Vero cells: ATCC® CCL-81™

[2]The material deposited by Dr. Gregory A. Dasch was grown in Minimum Essential Medium with Earle's salts (Invitrogen™ 10370-021) supplemented with 10% irradiated fetal bovine serum (Lonza 14-471F), 2 mM L-glutamine (Invitrogen™ 25030-081) and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 13 days at 34°C and 5% CO_2.

[3]Also consistent with other *Rickettsia* species

[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]7 days at 34°C and 5% CO_2 with media overlay

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

Date: 20 MAY 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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