

Certificate of Analysis for NR-333

Rickettsia rickettsii, Strain Smith

Catalog No. NR-333

(Derived from ATCC® VR-149™)

Product Description: Cell lysate and supernatant from African green monkey kidney (Vero) cells¹ infected with *Rickettsia rickettsii*, strain Smith.

Lot²: 58362753 Manufacturing Date: 08DEC2008

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Report results	Cell rounding and sloughing
Identification by Sequencing of Citrate Synthase Gene (~ 660 bp)	Identical to GenBank: CP000848 Rickettsia rickettsii	Identical to GenBank: CP000848 Rickettsia rickettsii
Titer by TCID ₅₀ Assay ^{3,4} in Vero Cells ¹	Report results	8.9 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 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 Brucella 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 (30-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹Vero cells: ATCC[®] CCL-81™

Date: 14 MAY 2009 **Signature:** Signature on file

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

Biodefense and Emerging Infections Research Resources Repository P.O. Box 4137 Manassas, VA 20108-4137 USA

Fax: 703-365-2898

800-359-7370

²Grown in Minimum Essential Medium with Earle's salts (Invitrogen™ 10370-021) supplemented with 10% irradiated fetal bovine serum (Lonza 14-471), 2 mM L-glutamine (Invitrogen™ 25030) and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 7 days at 37°C and 5% CO₂.

³The 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.

⁴5 days at 37°C and 5% CO₂ with media overlay

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