

***Rickettsia rickettsii*, Strain Bitterroot**

Catalog No. NR-393

(Derived from ATCC® VR-891™)

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

Lot²: 58362778

Manufacturing Date: 15SEP2008

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Report results	Cell rounding and sloughing
Identification by Sequencing of Citrate Synthase Gene (~ 980 bp)	Identical to GenBank: U59729 <i>Rickettsia rickettsii</i>	Identical to GenBank: U59729 <i>Rickettsia rickettsii</i>
Titer by TCID ₅₀ Assay ^{3,4} in Vero Cells ¹	Report results	8.9 X 10 ³ 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 No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth 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™

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 4 days at 34°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.

⁴6 days at 34°C and 5% CO₂ with media overlay

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

Date: 14 MAY 2009

Signature: Signature on file

Title: Technical Manager, BEI Authentication or designee

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