

Certificate of Analysis for NR-19221

Orientia chuto, Strain Dubai

Catalog No. NR-19221

(Derived from ATCC® VR-1744™)

Product Description: Mus musculus connective tissue fibroblast cells1 infected with Orientia

chuto, strain Dubai

Lot²: 60840747 Manufacturing Date: 19NOV2015

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in CCL-1 Cells ¹	Report results	Cell rounding
Identification by Sequencing of 16S Ribosomal RNA Gene (~ 799 bp)	Consistent with <i>Orientia</i> chuto, strain Dubai	100% identity with <i>Orientia</i> chuto, strain Dubai (GenBank: HM852447)
Titer by TCID₅₀ Assay³,⁴ in CCL-1 Cells¹ by PCR	Report results	2.3 × 10 ⁴ TCID ₅₀ per mL
PCR Amplification of Extracted DNA	~ 990 bp amplicon	~ 990 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 (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹NCTC Clone 929 cells (L-cell, L-929, derivative of strain L): ATCC[®] CCL-1™

Date: 01 MAR 2016

Signature:

BEI Resources Authentication

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.

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²Grown in Eagle's Minimum Essential Medium with Earle's salts, non-essential amino acids, L-glutamine and sodium pyruvate (ATCC[®] 30-2003) supplemented with 2% horse serum (ATCC[®] 30-2040) for 14 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.

⁴14 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