

Francisella tularensis subsp. tularensis, Strain SCHU S4 Δ*clpB*/Δ*capB*

Catalog No. NR-56772

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Product Description:

Francisella tularensis (*F. tularensis*) subsp. *tularensis*, strain SCHU S4 Δ*clpB*/Δ*capB* is a double-deletion mutant of genes *clpB*, encoding a heat shock gene, and *capB*, encoding a capsular polyglutamate biosynthesis protein (also referred to as FTT0805), from *F. tularensis* subsp. *tularensis*, strain SCHU S4. Strain SCHU S4 is a clone of highly virulent strain SCHU, which was isolated in 1941 from a human case of tularemia in Ohio, USA. NR-56772 was produced by inoculation of the deposited material into Mueller Hinton broth and grown for 3 days at 37°C in an aerobic atmosphere. Broth inoculum was added to Chocolate agar kolles, which were grown for 3 days at 37°C in an aerobic atmosphere to produce this lot.

Lot: 70052260

Manufacturing Date: 19SEP2022

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology Motility (wet mount) Biochemical characterization Catalase Oxidase Glucose Maltose Sucrose Glycerol	Gram-negative rods Report results Report results Positive Negative Report results Report results Report results Report results	Gram-negative rods Circular, convex, entire, smooth and gray Non-motile Positive Negative Negative Negative Negative
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1470 base pairs) Digital DNA-DNA hybridization (dDDH) ¹ Next-Generation Sequencing (NGS) analysis for confirmation of deleted genes <i>clpB</i> gene <i>capB</i> gene	≥ 99% sequence identity to <i>F. tularensis</i> subsp. <i>tularensis</i> , strain SCHU S4 (GenBank: AJ749949.2) ≥ 70% for species identification Deletion confirmed Deletion confirmed	100% sequence identity to <i>F. tularensis</i> subsp. <i>tularensis</i> , strain SCHU S4 (GenBank: AJ749949.2) <i>F. tularensis</i> (98.0%) Deletion confirmed ^{2,3} Deletion confirmed ⁴
Purity (post-freeze) 7 days at 37°C in an aerobic atmosphere with and without 5% CO ₂ on Tryptic Soy agar with 5% defibrinated sheep blood	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)	Growth	Growth

¹Relatedness between bacterial strains has traditionally been determined using DDH. For additional information, refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." *Stand. Genomic Sci.* 2 (2010): 117-134. PubMed: 21304684.

²*clpB* gene deletion was confirmed by NGS analysis of NR-56770 with the *F. tularensis* subsp. *tularensis*, strain SCHU S4 whole genome sequence (GenBank: AJ749949.2) spanning locus tag FTT_1768c (upstream region of *clpB*) and locus tag FTT_1770 (downstream of *clpB*), illustrating the deletion of 2,444 nucleotide deletion, approximately 95% of the total *clpB* gene, between the first 46 nucleotides and final 78 nucleotides of the protein, as described by the depositor (Conlan, J W., et al. "Differential Ability of Novel Attenuated Targeted Deletion Mutants of *Francisella tularensis* Subspecies *tularensis* Strain SCHU S4 to Protect Mice Against Aerosol Challenge with Virulent Bacteria: Effects of Host Background and Route of Immunization." *Vaccine* 28 (2010): 1824-1831. PubMed: 20018266.).

³Verification of the $\Delta c/pB$ mutation by whole genome sequencing (WGS) analysis and demonstration of significant attenuation in culture confirms that NR-56770 conforms to the criteria listed for exclusion of *Francisella tularensis* subsp. *tularensis*, strain SCHU S4 $\Delta c/pB$ from the requirements of 42 CFR part 73, i.e., the Select Agent guidelines, and is suitable for use in BSL2 laboratories.

⁴*capB* gene deletion was confirmed by NGS analysis of NR-56770 with AJ749949.2 spanning locus tag FTT_0804 (upstream region of *capB*) and locus tag FTT_0806 (downstream of *capB*).

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