

Certificate of Analysis for NR-4703

Yersinia pestis, Strain K25 Derivative 80 (D80)

Catalog No. NR-4703

Product Description: *Yersinia pestis* (*Y. pestis*) is an aerobic, non-spore-forming, Gram-negative rod-shaped bacterium. *Y. pestis*, strain K25(D80) is an avirulent derivative of the K25 strain that contains the pMT1 and pPCP1 plasmids, but lacks the pCD1 plasmid that is essential for virulence as well as the unstable chromosomal *pgm* locus.

Lot¹: 58324518 Manufacturing Date: 19SEP2008

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology ²	Report results	Circular, convex, entire, opaque (Figure 1)
Congo red (CR) agar ^{3,4}	No red colonies (crb ⁻)	No red colonies (crb ⁻)
Biochemical Analyses	, ,	, ,
Analytical profile index (API 20 E [®])	Consistent with Y. pestis	Consistent with Y. pestis
Nitrate reduction	Negative	Negative
Fermentation of glycerol	Positive	Positive
Urease	Negative	Negative
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1440 bp)	Consistent with Y. pestis ⁵	Consistent with Y. pestis ⁵
PCR Assay of Extracted DNA		
Y. pestis specific sequence (YPO0396) ⁶	~ 800 bp amplicon	~ 800 bp amplicon
16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Presence of virulence-associated plasmids		·
pMT1 (pFra; 100 kb plasmid)	~ 1200 bp amplicon	~ 1200 bp amplicon
pCD1 (pYV; 70 kb plasmid)	None detected	None detected
pPCP1 (pPla; 9.5 kb plasmid)	~ 400 bp amplicon	~ 400 bp amplicon
Viability (post-freeze) ²	Growth on agar	Growth on agar

Y. pestis, strain K25(D80) was deposited by Professor Robert R. Brubaker of the Department of Microbiology and Molecular Genetics at Michigan State University, East Lansing, Michigan. NR-4703 was prepared by Tryptic Soy Broth culture of the deposited material and grown 48 hours at 28°C and aerobic atmosphere. Broth inoculum was added to Kolles which were grown 48 hours at 28°C and aerobic atmosphere to produce this lot

Figure 1



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²48 hours at 28°C and aerobic atmosphere on Tryptic Soy Agar

³7 days at 28°C and aerobic atmosphere on CR agar

⁴Hare, J. M. and K. A. McDonough. "High-Frequency RecA-Dependent and -Independent Mechanisms of Congo Red Binding Mutations in *Yersinia pestis*." <u>J. Bacteriol.</u> 181 (1999): 4896-4904. PubMed: 10438760.

⁵Also consistent with other Yersinia species

⁶Sequence locus tag YPO0396 codes for an uncharacterized protein that is highly conserved in Y. pestis



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Date: 09 MAR 2011 **Signature:**

Title: Technical Manager, BEI Authentication or designee

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