

Certificate of Analysis for NR-4685

Yersinia pestis, Strain KIM Derivative 23 (D23)

Catalog No. NR-4685

Product Description: *Yersinia pestis* (*Y. pestis*) is an aerobic, non-spore-forming, Gram-negative rod-shaped bacterium. *Y. pestis*, strain KIM(D23) is an avirulent derivative that contains the pMT1, but lacks the pCD1 and pPCP1 plasmids that are essential for virulence as well as the unstable chromosomal *pgm* locus.

Lot¹: 58324515 Manufacturing Date: 10SEP2008

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology ²	Report results	Circular, convex, entire and 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 (~ 1420 bp)	Consistent with Y. pestis	Consistent with Y. pestis ⁵
	Identical to GenBank: AE009952	Identical to GenBank: AE009952
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)	None detected	None detected
Viability (post-freeze) ²	Growth on agar	Growth on agar

¹Y. pestis, strain KIM(D23) was deposited by Professor Robert R. Brubaker of the Department of Microbiology and Molecular Genetics at Michigan State University, East Lansing, Michigan. NR-4685 was prepared by broth (Tryptic Soy Broth; BD 211768) culture of the deposited material for 48 hours at 28°C and aerobic atmosphere.

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





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²48 hours at 28°C and aerobic atmosphere on Tryptic Soy Agar (BD 236950)

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

⁴Haré, 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



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Date: 04 NOV 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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