

Certificate of Analysis for NR-4697

Yersinia pestis, Strain K25 Derivative 72 (D72)

Catalog No. NR-4697

Product Description: Yersinia pestis (Y. pestis) is an aerobic, non-spore-forming, Gram-negative rod-shaped bacterium. Y. pestis, strain K25(D72) is an avirulent derivative of the K25 strain that contains the pMT1 and pPCP1 plasmids as well as the unstable chromosomal pgm locus, but lacks the pCD1 plasmid that is essential for virulence. This preparation contains two different colony morphologies that have been individually characterized and are shown to be identical in the assays utilized.

Lot¹: 57988654 Manufacturing Date: 16NOV2007

TEST	SPECIFICATIONS	RESULTS	
Phenotypic Analysis		Colony type 1	Colony type 2
Cellular morphology	Gram-negative rods	Gram-negative rods	Gram-negative rods
Colony morphology ²	Report results	Circular, convex, entire, opaque (Figure 1)	Circular, convex, entire, opaque, pinpoint (Figure 1)
Congo red (CR) agar ^{4,5}	Red colonies (Crb+)	Red colonies (Crb+)	Red colonies (Crb+)
Biochemical Analyses	,	, ,	, ,
Analytical profile index (API 20 E®)	Consistent with Y. pestis	Consistent with Y. pestis	Consistent with Y. pestis
Nitrate reduction	Negative	Negative	Negative
Fermentation of glycerol	Positive	Positive	Positive
Urease	Negative	Negative	Negative
Genotypic Analysis			
Sequencing of 16S ribosomal RNA gene (~ 1400 bp)	Consistent with Y. pestis	Consistent with Y. pestis ⁶	Consistent with Y. pestis ⁶
PCR Assay of Extracted DNA			
16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon	~ 1500 bp amplicon
Presence of virulence-associated plasmids	·		
pMT1 (pFra; ~ 100 kb plasmid)	~ 1200 bp amplicon	~ 1200 bp amplicon	~ 1200 bp amplicon
pCD1 (pYV; ~ 70 kb plasmid)	None detected	None detected	None detected
pPCP1 (pPla; ~ 9.5 kb plasmid)	~ 400 bp amplicon	~ 400 bp amplicon	~ 400 bp amplicon
Viability (post-freeze) ²	Growth on agar	Growth on agar	Growth on agar

Y. pestis, strain K25(D72) was deposited by Professor Robert R. Brubaker of the Department of Microbiology and Molecular Genetics at Michigan State University, East Lansing, Michigan. NR-4697 was prepared by broth (Tryptic Soy Broth; BD 211768) 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.

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

³Colony type 2 grows on top of colony type 1

⁴1 to 4 days at 28°C and aerobic atmosphere on CR agar

⁵Hare, J. M. and K. A. McDonough. "High-Frequency RecA-Dependent and -Independent Mechanisms of Congo Red Binding Mutations in *Yersinia pestis*." J. Bacteriol. 181 (1999): 4896-4904. PubMed: 10438760.

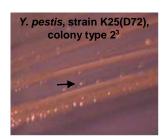
⁶Also consistent with other Yersinia species



Certificate of Analysis for NR-4697

Figure 1





Date: 13 AUG 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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E-mail: contact@beiresources.org

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