

Certificate of Analysis for NR-4694

Yersinia pestis, Strain Kimberley Derivative 12 (D12)

Catalog No. NR-4694

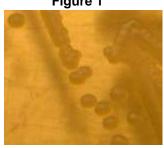
Product Description: *Yersinia pestis* (*Y. pestis*) is an aerobic, non-spore-forming, Gram-negative rod-shaped bacterium. *Y. pestis*, strain Kimberley(D12) is an avirulent derivative of the Kimberley strain that contains the pMT1 and pPCP1 plasmids as well as the unstable *pgm* locus, but lacks the pCD1 plasmid that is essential for virulence.

Lot¹: 57753204 Manufacturing Date: 20JUL2007

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rod	Gram-negative rod
Colony morphology ²	Report results	Circular, convex, entire, opaque (Figure 1)
Congo red (CR) agar ^{3,4}	Red colonies (Crb+)	Red colonies (Crb+)
Biochemical Analyses	,	
Analytical profile index (API 20 E®)	Consistent with Y. pestis	Consistent with Y. pestis
Nitrate reduction	Positive	Positive
Fermentation of glycerol	Negative	Negative
Urease	Negative	Negative
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (1420 bp)	Consistent with Y. pestis	Consistent with Y. pestis ⁵
PCR Assay of Extracted DNA		
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) ⁴	Growth on agar	Growth on agar

¹Y. pestis, strain Kimberley(D12) was deposited by Professor Robert R. Brubaker of the Department of Microbiology and Molecular Genetics at Michigan State University, East Lansing, Michigan. NR-4694 was prepared by broth (Trypticase Soy Broth; BD 211768) culture of the deposited material. 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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NR-4694 57753204 04SEP2008

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

³²⁴ hours 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*." J. Bacteriol. 181 (1999): 4896-4904. PubMed: 10438760.

⁵Also consistent with other Yersinia species



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

Title: Technical Manager, BEI Authentication or designee

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NR-4694 57753204 04SEP2008