

## **Certificate of Analysis for NR-4689**

## Yersinia pestis, Strain A12 Derivative 6 (D6)

## Catalog No. NR-4689

**Product Description:** *Yersinia pestis* (*Y. pestis*) is an aerobic, non-spore-forming, Gram-negative rod-shaped bacterium. *Y. pestis*, strain A12(D6) is an avirulent derivative that lacks two of the three plasmids found in typical *Y. pestis* strains, the pCD1 plasmid that is essential for virulence and the pPCP1 plasmid, as well as the chromosomal virulence-associated locus *pgm*. Strain A12(D6) contains the pMT1 plasmid.

Lot<sup>1</sup>: 58098884 Manufacturing Date: 07MAR2008

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rod	Gram-negative rod
Colony morphology <sup>2</sup>	Report results	Circular, convex, entire, opaque (Figure 1)
Congo red (CR) agar <sup>3</sup>	No red colonies	No red colonies
Biochemical Analyses		
Analytical profile index (API 20 E®)	Consistent with Y. pestis	Consistent with Y. pestis
Nitrate reduction <sup>4</sup>	Positive	Negative
Fermentation of glycerol	Negative	Negative
Urease	Negative	Negative
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1430 bp)	Consistent with Y. pestis	Consistent with Y. pestis <sup>5</sup>
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Presence of virulence-associated plasmids	i i	' '
pMT1 (pFra; 110 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) <sup>2</sup>	Growth on agar	Growth on agar

¹Y. pestis, strain A12(D6) was deposited by Professor Robert R. Brubaker of the Department of Microbiology and Molecular Genetics at Michigan State University, East Lansing, Michigan. NR-4689 was prepared by broth (Tryptic Soy Broth; BD 211768) culture of the deposited material.
²48 hours at 28°C and aerobic atmosphere on Tryptic Soy Agar.

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<sup>&</sup>lt;sup>3</sup>7 days at 28°C and aerobic atmosphere on CR agar.

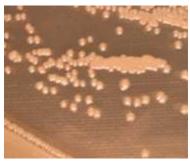
<sup>&</sup>lt;sup>4</sup>NR-4689 was deposited as biovar Orientalis which is published as positive for nitrate reduction and negative for glycerol fermentation (Zhou, D., et al. "Genetics of Metabolic Variations Between *Yersinia pestis* Biovars and the Proposal of a New Biovar, Microtus." <u>J. Bacteriol.</u> 186 (2004): 5147-5152. PubMed: 15262951). Our nitrate reduction assay was performed twice and the results were negative on both occasions. There are currently no biovars associated with negative nitrate reduction and negative glycerol fermentation.

<sup>&</sup>lt;sup>5</sup>Also consistent with other Yersinia species.



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**Date:** 31 JUL 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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