

***Yersinia pestis*, Strain PH 80/63 (NCTC 10329), Heat-Inactivated**

Catalog No. NR-51667

Product Description: NR-51667 is a preparation of *Yersinia pestis* (*Y. pestis*), strain PH 80/63 that has been inactivated by heating to 95°C for 20 minutes.

Lot¹: 70025951

Manufacturing Date: 04MAR2019

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Pre-inactivation Post-inactivation	Gram-negative rods No viable bacteria detected	Gram-negative rods No viable bacteria detected
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1470 base pairs) Pre-inactivation Post-inactivation	≥ 99% sequence identity to <i>Y. pestis</i> type strain NCTC 5923 (GenBank: NR_025160.1) ≥ 99% sequence identity to <i>Y. pestis</i> , strain PH 80/63 (NR-36154)	100% sequence identity to <i>Y. pestis</i> type strain NCTC 5923 (GenBank: NR_025160.1) 100% sequence identity to <i>Y. pestis</i> , strain PH 80/63 (NR-36154)
Functional Activity by PCR Pre-inactivation Post-inactivation	700 to 1500 base pair amplicon 700 to 1500 base pair amplicon	~ 1500 base pair amplicon ~ 1500 base pair amplicon
SDS-PAGE Analysis (Coomassie Blue Staining)	Protein profile similar to active <i>Y. pestis</i>	Protein profile similar to active <i>Y. pestis</i> (Figure 1) ²
Functional Activity by Western Blot Analysis Antibody to YopM protein, clone CE 15-1 Antibody to YopM protein, clone 2A3.3A8.1A2 Antibody to LcrV protein Antiserum to F-1 antigen	Reactive Reactive Reactive Reactive	Reactive (Figure 2) ^{2,3} Reactive (Figure 3) ^{2,4} Reactive (Figure 4) ^{2,5} Reactive (Figure 5) ^{2,6}
Purity (post-freeze)⁷	No growth	No growth
Bacterial Inactivation (14 days) 10% of heat-inactivated preparation plated on agar ⁸	No viable bacteria detected	No viable bacteria detected

¹NR-51667 was inactivated by heating *Y. pestis*, strain PH 80/63 to 95°C for 20 minutes. The material was cooled to room temperature, vialled and frozen to produce this lot. The material used for inactivation was produced from the second passage of BEI Resources NR-36154 lot 61427721, grown under propagation conditions.

²Results were similar with untreated *Y. pestis*, strain KIM Derivative 19 (D19) (BEI Resources NR-4681 lot 70012951).

³Using a 1:500 dilution of Monoclonal Anti-*Y. pestis* Outer Protein M (YopM), Clone 2A3.3A8.1A2 (produced *in vitro*) (BEI Resources NR-799) as a primary antibody and a 1:2000 dilution of goat anti-mouse IgG with HRP conjugate as a secondary antibody

⁴Using a 1:500 dilution of Monoclonal Anti-*Y. pestis* Outer Protein M (YopM), Clone CE 15-1 (produced *in vitro*) (BEI Resources NR-800) as a primary antibody and a 1:2000 dilution of goat anti-mouse IgG with HRP conjugate as a secondary antibody

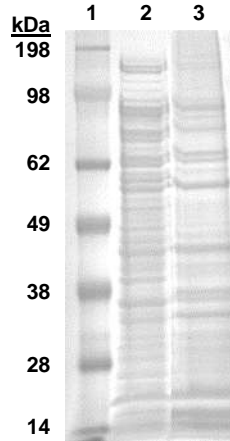
⁵Using a 1:500 dilution of Monoclonal Anti-*Y. pestis* LcrV Protein (produced *in vitro*) (BEI Resources NR-3831) as a primary antibody and a 1:2000 dilution of goat anti-mouse IgG with HRP conjugate as a secondary antibody

⁶Using a 1:500 dilution of Polyclonal Anti-*Y. pestis* F1-Antigen (antiserum, Goat) (BEI Resources NR-31024) as a primary antibody and a 1:2000 dilution of rabbit anti-goat IgG with HRP conjugate as a secondary antibody

⁷Purity of this lot was assessed for 14 days at 37°C in an aerobic atmosphere with 5% CO₂ in Tryptic Soy broth and on Tryptic Soy agar with and without 5% defibrinated sheep blood.

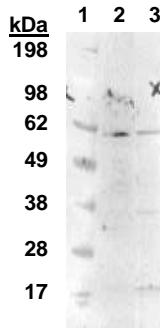
⁸NR-51667 was incubated 14 days under propagation conditions. The inactivation procedure that was used has been shown to consistently inactivate 100% of *Yersinia* spp.

Figure 1: SDS-PAGE Analysis of Heat-Inactivated *Y. pestis*, strain PH 80/63 (NR-51667)



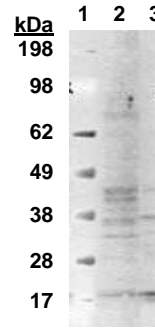
Lane 1: SeeBlue™ Plus2 Pre-Stained Protein Standard (Invitrogen™; 6 µL)
 Lane 2: NR-4681 (26 µL)
 Lane 3: NR-51667 (26 µL)

Figure 2: Western Blot with *Y. pestis* YopM mAb (NR-799)



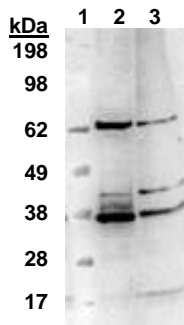
Lane 1: SeeBlue™ Plus2 Pre-Stained Protein Standard (6 µL)
 Lane 2: NR-4681 (26 µL)
 Lane 3: NR-51667 (26 µL)

Figure 3: Western Blot with *Y. pestis* YopM mAb (NR-800)



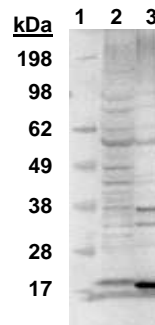
Lane 1: SeeBlue™ Plus2 Pre-Stained Protein Standard (6 µL)
 Lane 2: NR-4681 (26 µL)
 Lane 3: NR-51667 (26 µL)

Figure 4: Western Blot with *Y. pestis* LcrV mAb (NR-3831)



Lane 1: SeeBlue™ Plus2 Pre-Stained Protein Standard (6 µL)
 Lane 2: NR-4681 (26 µL)
 Lane 3: NR-51667 (26 µL)

Figure 5: Western Blot with *Y. pestis* F-1 mAb (NR-31024)



Lane 1: SeeBlue™ Plus2 Pre-Stained Protein Standard (6 µL)
 Lane 2: NR-4681 (26 µL)
 Lane 3: NR-51667 (26 µL)

/Heather Couch/

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