Polyclonal Anti-*Francisella tularensis* Pathogenicity Determinant D (PdpD) Protein (antiserum, Rabbit)

Catalog No. NR-4377

For research use only. Not for human use.

Contributor:
Francis E. Nano, Ph.D., Professor, Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada

Product Description:
Rabbit polyclonal antibody to a recombinant protein fragment of the pathogenicity determinant D protein (PdpD) of *Francisella tularensis* was produced in rabbit. The PdpD protein fragment contained a C-terminal His-tag for purification and was expressed in *Escherichia coli*. Purified antigen was injected into two rabbits and serum from both rabbits was pooled to produce NR-4377.

Two large convergently transcribed operons, *pdpDiglABCD* and *pdpA*, are encoded by the *Francisella* pathogenicity island, which harbor genes necessary for intramacrophage growth and virulence in mice. The only gene that is not required for pathogenicity is *pdpD*, although it may still contribute to intramacrophage growth and virulence.[1] The presence or absence of *pdpD* may account for the differences in virulence between type A and B strains, since *pdpD* is only present in type A strains. The *pdpD* gene is a member of the *pdpDiglABCD* operon and is translated to an approximately 140 kDa protein.[2,3]

Material Provided:
Each vial contains approximately 1 mL of NR-4377.

Packaging/Storage:
NR-4377 was packaged aseptically in screw capped plastic cryovials. The product is provided frozen and should be stored at -20°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Functional Activity:
NR-4377 has been shown to be reactive with the PdpD protein of wild-type *Francisella tularensis* using Western blot analysis.

Proper antigen preparation for Western blotting is extremely important as PdpD is produced in very small quantities in the organism. Preparation of a sarkosyl insoluble membrane fraction will enrich PdpD sufficiently to visualize. See Appendix I below for instructions for preparation of the sarkosyl insoluble membrane fraction. A positive control, such as *F. tularensis* subsp. *novicida* U112 (BEI Resources NR-13), and a negative control, such as *F. tularensis* subsp. *novicida* JL12 (BEI Resources NR-583), should be used when attempting Western blot analysis of PdpD for the first time.

Note: NR-4377 is not recommended for use in ELISA.

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Polyclonal Anti-*Francisella tularensis* Pathogenicity Determinant D (PdpD) Protein (antiserum, Rabbit), NR-4377."

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References:

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2. Nano, F. E., et al. "A *Francisella tularensis* Pathogenicity Island Required for Intramacrophage Growth." J. Bacteriol. 186 (2004): 6430–6436. PubMed: 15375123.
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Appendix I: Preparation of Sarkosyl Insoluble Membrane Fraction

1. Grow *F. tularensis* subsp. *novicida* U112 (100 mL) overnight at 37°C.
2. Pellet the cells and resuspend in 30 mL of PBS.
3. Mechanically shear the cells by three passages through a French Pressure Cell (American Instruments Co., Silver Spring, MD) at 1,250 PSI.
4. Centrifuge the pressed cells at 10,000 X *g* for 15 min at 4°C to remove unbroken cells. Remove a small sample of the supernatant as the total protein fraction.
5. Separate the lysate into the membrane fraction and the soluble protein fraction by ultracentrifugation for 1 h at 100,000 X *g* at 4°C in a Beckman Type 45 Ti rotor.
6. Resuspend the membrane pellet in 2 mL of 1% *N*-lauroyl sarcosine (Sigma).
7. Separate the sarkosyl soluble (inner membrane) and insoluble (outer membrane) fractions by ultracentrifugation for 1 h at 100,000 X *g* at 4°C in a Beckman TLA-100.3 micro-ultracentrifuge.

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