Monoclonal Anti-*Francisella tularensis* Pathogenicity Determinant A (PdpA) Protein, Clone PdpA1 (produced *in vitro*)

Catalog No. NR-3197

For research use only. Not for human use.

Contributor:

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Product Description:

Antibody Class: IgG1
Mouse monoclonal antibody specific to a histidine-tagged recombinant fragment of the pathogenicity determinant A protein of *Francisella tularensis* was produced *in vitro*.

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.[1] PdpA is an approximately 95 kDa protein that is required for intracellular bacterial replication and may be important for the escape of *Francisella* species from the phagosome to the cytosol.[2]

Material Provided:

Each vial contains approximately 1 mL of NR-3197 in Dulbecco's Modified Eagle's Medium supplemented with 5% fetal bovine serum. The estimated concentration, expressed as mg per mL, is shown on the Certificate of Analysis.

Packaging/Storage:

NR-3197 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-3197 has been shown to be specific for the PdpA protein of wild-type *Francisella tularensis* using Western blot analysis. Please see Appendix I below for Western blotting instructions.

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: Monoclonal Anti-*Francisella tularensis* Pathogenicity Determinant A (PdpA) Protein, Clone PdpA1 (produced *in vitro*), NR-3197."

Disclaimers:

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References:

1. Barker, J. R. and K. E. Klose. "Molecular and Genetic Basis of Pathogenesis in *Francisella tularensis*." Ann. N. Y. Acad. Sci. Mar 29 2007 (Epub ahead of print). PubMed: 17395737.
2. Mariathasan, S., et al. "Innate Immunity Against *Francisella tularensis* is Dependent on the ASC/Caspase-1 Axis." J. Exp. Med. 202 (2005): 1043–1049. PubMed: 16230474.
3. Gray, C. G., S. C. Cowley, K. K. Cheung, and F. E. Nano. "The Identification of Five Genetic Loci of *Francisella novicida* Associated with Intracellular Growth." FEMS Microbiol. Lett. 215 (2002): 53–56. PubMed: 12393200.

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4. Nano, F. E., et al. "A *Francisella tularensis* Pathogenicity Island Required for Intramacrophage Growth." J. Bacteriol. 186 (2004): 6430–6436. PubMed: 15375123.
5. Cowley, S. C., C. J. Gray, and F. E. Nano. "Isolation and Characterization of *Francisella novicida* Mutants Defective in Lipopolysaccharide Biosynthesis." FEMS Microbiol. Lett. 182 (2000): 63–67. PubMed: 10612732.

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Appendix 1: Western Blotting Protocol for PdpA

Plate grown sample:

1. Heavily streak a plate with desired strain and grow up in desired conditions
2. Scrape up and suspend cells in 1 mL of PBS with bacterial protease inhibitor (Sigma P8465)
3. Skip to step 6

Broth Grown Sample:

1. Inoculate 5 mL of desired strain into Tryptic Soy Broth (BD211825) and let grow without shaking overnight
2. Subculture 2 mL of the overnight culture into 20 mL of new Tryptic Soy Broth and grow with shaking for ~ 4 hours (150–170 Klett units). If adding Desferal (an iron chelator) let the cultures grow for 1 hour, add Desferal to one culture, and incubate both cultures for an additional 3 hours
3. Spin cells down at 3500 rpm for 15 minutes
4. Resuspend the pellet in 1 mL of PBS with bacterial protease inhibitors (Sigma P8465)
5. Continue to step 6

From step 6 onwards processing of both sample types is the same:

6. Sonicate the cells in 5 bursts of 30 seconds (you should be able to visualize the cell clarity after sonication; if the cell suspension is still very opaque the sample is too concentrated and should be diluted with some PBS)
   Note: Sonication is not absolutely necessary but sometimes generates protein bands that are sharper.
7. After sonication determine protein levels using desired assay (if using the Bradford method the samples assay well when diluted 10-fold)
8. Prepare samples for SDS-PAGE
9. Run 15 µg of total protein per lane on 7% gels (Use a Broad Range Protein Ladder (NEB P7708S or equivalent) and run all but the 175, 85 and 62 kDa markers off the gel)
10. Transfer the protein onto Immobilon-FL (Millipore) PVDF for 45 minutes (use the BioRad wet transfer or similar system)
11. Block the membrane in PBS with 5% Skim milk for at least 30 minutes
12. Add anti-PdpA monoclonal antibody at 1:500 and incubate at least 4 hours to overnight
13. Visualize bound antibody using desired method.

Note: If there is a problem visualizing PdpA, try using the *Francisella* lipopolysaccharide (LPS) mutant SC92 (NR-578, see reference 5 for more information). Alternatively, add urea to the protein sample to a final concentration of 8M at step 8 to dissociate the proteins from LPS.

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