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## PG-PS 100P

Rev 4/98

### A. Product Name

- PG-PS 100P

### B. Catalog Number

- 210868

### C. Intended Use

- **PG-PS 100P** is used in various animal models for the induction of acute inflammatory responses which can evolve into a remittent, chronic, inflammatory response. Experimental chronic diseases induced by PG-PS in rats include chronic, remittent, erosive arthritis, granulomatous enterocolitis (resembling Crohn's disease), granulomatous hepatitis, intestinal hemorrhage, carditis and vasculitis. PG-PS induces animal responses which are representative of naturally caused inflammatory disorders. PG-PS 100P is used most commonly in the rat intra-articular (IA) injection model for arthritis, described below.

### D. Product Description

- **PG-PS 100P** consists of purified peptidoglycan-polysaccharide polymers which are isolated from the sonicated cell wall of *Streptococcus pyogenes*, Group A, D58 strain. The peptidoglycan is the primary immunogenic moiety. The polysaccharide, when bound to this peptidoglycan moiety, allows for the chronic inflammation seen in animal models by protecting this moiety from degradation. The PG-PS 100P is supplied as a white, opalescent liquid suspension in sterile 0.85% saline. The rhamnose concentration of the product is 5 to 8 mg/ml and the MW of the product is approximately  $5 \times 10^7$  Daltons.
- **PG-PS 100P** is assayed in our laboratories using the rat model described below.

### E. Precautions

- The PG-PS 100P material must be stored and handled in an aseptic manner to avoid product contamination. Such contamination may affect animal model results.
- **For Research Use Only.** Not for use in diagnostic procedures.

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### F. Storage

- 2 - 8°C
- Discard any reagent which has become obviously contaminated or discolored.

### G. **INSTRUCTIONS FOR USE** - Procedure for Arthritic Rat Model, Intra-articular Injection

<b>Materials Provided</b>	PG-PS 100P, 5 - 8 mg rhamnase/ml
<b>Materials Required</b>	Lewis Strain Rats, female, 150 - 200 gm each
	Sterile 0.85% saline
	Balance
	Vortexer
	Caliper
	26 gauge needles
	Pipettor

1. Randomly divide the rats into control and assay groups as required for the model. Label each group of rats appropriately. Control groups are defined as those rats receiving saline (negative control) and those rats receiving PG-PS 100P (positive control) with no other intervention or treatment such as anti-inflammatory drugs. Assay groups are defined as rats receiving PG-PS 100P with intervention.
2. Weigh each rat in each group and determine the average body weight of each group. This average must be between 150 - 200 gm.
3. Measure each rat's ankle (maximal lateral) individually with a caliper to determine the base line ankle measurement. Measure both left and right ankles. Each ankle should be measured 3 times and averaged.
4. Vortex the PG-PS 100P for 30 seconds to thoroughly mix the material. Suspensions which appear to no longer be smooth or uniform (aggregated suspension) should be sonicated at low energy levels for 10-20 seconds with a **probe** type sonicator prior to injection.

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5. Dilute the PG-PS 100P to a concentration of 400 - 500  $\mu\text{g}/\text{ml}$  using sterile 0.85% saline.
6. Anesthetize each rat in the assay and positive control groups.
7. Administer 10  $\mu\text{l}$  of diluted PG-PS 100P to each ankle in the rats in both the assay and positive control groups using an intra articular (IA) injection route, for a total dose of 4 - 5  $\mu\text{g}$  rhamnose per ankle. The IA injection is delivered through the Achilles tendon just proximal to the calcaneus. A pipettor with a 26 gauge tuberculin needle attached to a modified tip works well for this type of injection.
8. Repeat steps # 6 and # 7 above for the negative control group of rats, except substitute sterile 0.85% saline for PG-PS 100P reagent.
9. Monitor the response for each rat group every day for the first six days post injection, then at least every three days for 24 days.
10. At 30 days post the initial IA injection and prepare 75  $\mu\text{g}/\text{ml}$  PG-PS 100P suspension in sterile 0.85% saline. Anesthetize the assay and positive control groups. Administer 0.4 ml (total dose 30  $\mu\text{g}$  rhamnose) intravenously (IV) into the tail vein of each animal.
11. Repeat step # 10 above for the negative control group of rats, except substitute sterile 0.85% saline for PG-PS 100P reagent.
12. Monitor the response for each rat group every day for the first six days post the reactivation injection, then at least every three days for 14 days.

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13. A reading of the response consists of 3 measurements with a caliper per ankle as originally measured in Step # 3 above. Each group of rat ankle measurements are averaged and recorded as one data point to produce a graphical representation of the data.

### H. Limitations of the Procedure

- Certain animals may not respond to the initial PG-PS 100P injection and are termed non-responders. This is typically due to the placement of the 100P material, and is not reflective of the quality of the product. Typically, 20% or less should be non-responders (in the positive control group) to consider the placement technique valid. Remove non-responders from the protocol after the initial 6 days post-injection and do not include this data in calculating averages or percent response.

### I. Expected Values

- Both an acute and chronic reactivation response must be evident in the PG-PS control group of rats. An acute response consists of a sharp increase in ankle measurements, typically 15% above baseline measurements. This rise reaches a peak in 1 - 2 days and is followed by a decline in the measurements on subsequent days. Reactivation will cause a rise in the ankle measurements (typically 15% above baseline), which should remain elevated for up to one week.
- No response should be observed in the negative (saline) control group of rats

### J. Related Literature List

1. **Abdulla, E. M., and J. H. Schwab.** 1965. Immunological Properties of bacterial cell wall mucopeptides. *Proc. Soc. Exp. Biol. Med.* **118**:359-362.
2. **Abdulla, E. M. and J. H. Schwab.** 1966. Biological properties of streptococcal cell wall particles. III. Dermonecrotic reaction to cell wall mucopeptides. *J. Bacteriol.* **91**:374-383.
3. **Bristol-Rothstein, L. A., and J. H. Schwab.** 1992. Bone-resorbing activity is expressed by rat macrophages in response to arthropathic streptococcal cell wall polymers. *Inflammation* **16**:485-496.
4. **Chetty, C., R. R. Brown, and J. H. Schwab.** 1983. Edema producing activity of Group A Streptococcal polysaccharide and its possible role in the pathogenesis of cell wall-induced polyarthritis. *J. Exp. Med.* **157**:1089-1100.
5. **Chetty, C., D. G. Klapper, and J. H. Schwab.** 1982. Soluble peptidoglycan-polysaccharide fragments of the bacterial cell wall induce acute inflammation. *Infect. Immun.* **38**:1010-1019.

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6. **Cromartie, W. J., and J. G. Craddock.** 1966. Rheumatic-like cardiac lesions in mice. *Science* **154**:285-288.
7. **Cromartie, W. J., J. G. Craddock, J. H. Schwab, S. K. Anderlie, and C. Yang.** 1977. Arthritis in rats after systemic injection of streptococcal cells or cell walls. *J. Exp. Med.* **146**:1585-1602.
8. **Dallorf, F. G., S. K. Anderle, R. R. Brown, and J. H. Schwab.** 1988. Mast cell activation by Group A streptococcal polysaccharide in the rat and its role in experimental arthritis. *A. J. Pathol.* **132**:258-264.
9. **Esser, R. E., S. K. Anderle, C. Chetty, S. A. Stimpson, W. J. Cromartie, and J. H. Schwab.** 1986. Comparison of Inflammatory reactions induced by intra articular injection of bacterial cell wall polymers. *A. J. Pathol.* **122**:323-334.
10. **Esser, R. E., J. H. Schwab, and R. A. Eisenberg.** 1985. Immunology of peptidoglycan-polysaccharide polymers from cell walls of Group A Streptococci, Pages 91-118. D. E. S. Stewart-Tull and M. Davies (ed.), *Immunology of the bacterial cell envelope.* John Wiley & Sons, Inc., New York.
11. **Esser, R. E., S. A. Stimpson, W. J. Cromartie, and J. H. Schwab.** 1985. Reactivation of streptococcal cell wall-induced arthritis by homologous and heterologous cell wall polymers. **28**:1402-1411.
12. **Fox, A., R. R. Brown, S. K. Anderlie, C. Chetty, W. J. Cromartie, H. Gooder, and J. H. Schwab.** 1982. Anthropathic properties related to the molecular weight of peptidoglycan-polysaccharide polymers of streptococcal cell walls. **35**:1003-1010.
13. **Fulghum, R. S., R. R. Brown, and J. H. Schwab.** 1995. PG-PS (Peptidoglycan) causes otitis Media (OM) in chinchillas. Abstracts of the General Meeting of the American Society for Microbiology. **95**:241.
14. **Greenblatt, J. J., R. J. Boackle, and J. H. Schwab.** 1978. Activation of the alternate complement pathway by peptidoglycan from streptococcal cell wall. *Infect. Immun.* **19**:296-303.
15. **Koga, T., K. Kakimoto, T. Hirofuji, S. Kotani, H. Ohkuni, K. Watantabe, N. Okada, H. Okada, A. Sumiyoshi, and K. Saisho.** 1985. Acute joint inflammation in mice after systemic injection of cell wall, its peptidoglycan, and chemically defined peptidoglycan subunits from various bacteria. *Infect. Immun.* **50**:27-34.
16. **Lichtman, S. N., S. Bachmann, E. E. Bender, L.C. Holt, J. H. Schwab, R. B. Sartor, and J. J. Lemasters.** 1991. Transplantation of livers from PG-PS treated rats causes reactivation of arthritis. *Gastroenterology* **100**:A766.
17. **Lichtman, S. N., S. Bachmann, S. R. Munoz, J. H. Schwab, D. E. Bender, R. B. Sartor, and J. J. Lemasters.** 1993. Bacterial cell wall polymers (peptidoglycan-polysaccharide) cause reactivation of arthritis. *Infect. Immun.* **61**:4645-4653.
18. **Lichtman, S. N., J. Wang, R. B. Sartor, C. Zhang, D. Bender, F. G. Jdalldorf, and J. H. Schwab.** Reactivation of arthritis induced by small bowel bacterial overgrowth in rats: role of cytokines, bacteria, and bacterial polymers. *Infect Immun.* **63**:2295-2301.
19. **Lichtman, S. N., J. Wang, J. H. Schwab, and J. J. Lemasters.** 1994. Comparison of peptidoglycan-polysaccharide and lipopolysaccharide stimulation of Kupffer cells to produce tumor necrosis factor and interleukin. *Hepatology.* **19**:1013-1022.

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20. **Regan, D. R., P. L. Cohen, W. J. Cromartie, and J. H. Schwab.** 1988. Immunosuppressive macrophages induced by arthropathic peptidoglycan-polysaccharide polymers from bacterial cell walls. *Clin. Exp. Immunol.* **74**:365-370.
21. **Sartor, R. B.** 1993. Role of the intestinal microflora in the pathogenesis and complications of inflammatory bowel disease, p. 175-187. *In* J. Scholmerich (ed.), *Inflammatory bowel disease pathophysiology as basis of treatment*. Kluwer Academic Publishers, Lancaster, United Kingdom
22. **Sartor, R. B., S. K. Anderle, W. J. Cromartie, and J. H. Schwab.** 1986. Localized gut-associated lymphoid tissue hemorrhage induced by intravenous peptidoglycan-polysaccharide polymers. *Infect. Immun.* **51**:521-528.
23. **Sartor, R. B., T. M. Bond, and J. H. Schwab.** 1988. Systemic uptake and intestinal inflammatory effects of luminal bacterial cell wall polymers in rats with acute colonic injury. *Infect. Immun.* **56**:2101-2108.
24. **Sartor, R. B., D. R. Cleland, C. J. Catalano, and J. H. Schwab.** 1985. Serum antibody to bacterial cell wall-peptidoglycan in inflammatory. *Gastroenterology.* **88**:1571.
25. **Sartor, R. B., W. J. Cromartie, D. W. Powell, and J. H. Schwab.** 1985. Granulomatous enterocolitis induced in rats by purified bacterial cell wall fragments. *Gastroenterology* **89**:587-595.
26. **Schwab, J. H.** 1982. Immune dysfunction associated with arthritis induced by peptidoglycan-polysaccharide polymers from streptococcal cell walls, p. 84-93. *In* Y. Yamamura, and S. Kotani (ed.), *Immunomodulation by microbial products and related synthetic compounds*. Excerpta Medica, Amsterdam.
27. **Schwab, J. H.** 1993. Bacterial cell wall arthritis: models of chronic recurrent polyarthritis and reactivation of monoarticular arthritis, B. Henderson, R. Pettifer, and J. Edwards (ed.), *Mechanisms and models in rheumatoid arthritis*, in press. Academic Press. Ltd., London.
28. **Schwab, J. H.** 1993. Phlogistic properties of peptidoglycan-polysaccharide polymers from cell walls of pathogenic and normal-flora bacteria which colonize humans. *Infect Immun.* **61**:4535-4539.
29. **Schwab, J. H., S. K. Anderle, R. R. Brown, F. G. Dalldorf, and R. C. Thompson.** 1991. Pro and anti-inflammatory roles of interleukin-1 recurrence of bacterial cell wall-induced arthritis in rats. *Infect. Immun.* **59**:4436-4442.
30. **Schwab, J. H., R. R. Brown, S. K. Anderlie, and P. M. Schlievert.** 1993. Superantigen can reactivate bacterial cell wall-induced arthritis. *The Journal of Immunology.* **150**:4151-4159.
31. **Schwab, J. H., W. J. Cromartie, S. H. Ohanian, and J. G. Craddock.** 1967. Association of experimental chronic arthritis with the persistence of Group A Streptococcal cell walls in the articular tissue. *J. Bacteriol.* **94**:1728-1735.
32. **Severijnen, A. J., M. P. Hazenberg, and J. P. Van de Merwe.** 1988. Induction of chronic arthritis in rats by cell wall fragments of anaerobic coccoid rods isolated from the faecal flora of patients with Crohn's disease. *Digestion* **39**:118-125.

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33. **Stein, H., R. Yarum, S. Levine, T. Dishon, and I. Ginsburg.** 1973. Chronic self-perpetuating arthritis induced in rabbits by a cell-free extract of Group A Streptococci. *Proc. Soc. Exp. Biol. Med.* **143**:1106-1112.
34. **Stimpson, S. A., R. R. Brown, S. K. Anderie, K. G. Klapper, R. L. Clark, W. J. Cromartie, and J. H. Schwab** 1986. Arthropathic properties of cell wall polymers from normal flora bacteria. *Infect. Immun.* **51**:240-249.
35. **Stimpson, S. A., and J. H. Schwab.** 1989. Chronic remittent erosive arthritis induced by bacterial peptidoglycan-polysaccharide structures, P. 381-394. J. Y. Chang, and A. J. Lewis (ed.), *Pharmacological methods in the control of inflammation.* Alan R. Liss, Inc., New York.
36. **Stimpson, S. A., J. H. Schwab, M. J. Janusz, S. K. Anderlie, R. R. Brown, and W. J. Cromartie.** 1986. Acute and chronic inflammation induced by peptidoglycan structures and polysaccharide complexes. p.273-290. P. H. Seidl and K. H. Schleifer (ed.), *Biological properties of peptidoglycan.* Walter de Gruyter & C., Berlin.
37. **Takada, H., and S. Kotani.** 1985. Immunopharmacological activities of synthetic muramyl-peptides, p. 119-152. D. E. S. Stewart-Tull and M. Davies (ed.), *Immunology of the bacterial cell envelope.* John Wiley & Sons, Inc., New York.
38. **Wahl, S. M., D. A. Hunt, J. B. Allen, R. L. Wilder, L. Paglia, and A. R. Hand.** 1986. Bacterial cell wall-induced hepatic granulomas. An in vivo model of T cell-dependent fibrosis. *J. Exp. Med.* **163**:884-902.
39. **Wells, A., G. Parajasegaram, M. Baldwin, C. Yang, M. Hammer, and A. Fox.** 1986. Uveitis and arthritis induced by systemic injection of streptococcal cell walls. *Invest. Ophthalmol.* **27**:109-115.
40. **Wilder, R. L., J. P. Case, L. J. Crofford, G. K. Kumkumian, R. Lafyatis, E. F. Remmers, H. Sano, E. M. Sternberg, and D. E. Yocum.** 1991. Endothelial cells and the pathogenesis of rheumatoid arthritis in humans and streptococcal cell wall arthritis in Lewis rats. *J. Cell. Biochem.* **45**:162-166.
41. **Yamada, T., C. Abell, M. Grisham.** 1993. Colonic hyperemia induced by bacterial cell wall polymers potential role of nitric oxide. **104**:A291.
42. **Yamada, T., R. B. Sartor, S. Marshall, R. D. Specian, and M. Grisham.** 1993. Mucosal injury and inflammation in a model of chronic granulomatous colitis in rats. **104**:759-771.