

Product Information

Lipid A, diphosphoryl from *Escherichia coli* F583 (Rd mutant)

Product Number **L 5399**
Storage Temperature 2-8 °C

Product Description

This product is the diphosphoryl form of Lipid A prepared from a rough strain *E. coli* lipopolysaccharide using treatment with mild acid and heat followed by chromatography.¹

Lipid A is a glucosamine disaccharide with a β (1 \rightarrow 6) linkage to which are attached two phosphate groups at positions 1 and 4'. Lipid A contains up to 7 fatty acid side chains. The approximate (or average) molecular weight is 1.7-1.8 kDa, depending on the number and identity of fatty acid chains present. The fatty acid composition will vary depending upon the method of production. The KDO (2-keto-3-deoxyoctonate) attachment was through the 6' position.

Lipopolysaccharides are composed of a hydrophobic lipid (lipid A), a hydrophilic core polysaccharide chain, and a hydrophilic O-antigenic polysaccharide side chain. Removal by hydrolysis of the polysaccharide chains from LPS produces Lipid A, either as the naturally occurring, cytotoxic diphosphoryl form¹ or the less toxic monophosphoryl form.^{2,3} The longer the polysaccharide chain is, the longer and more difficult the hydrolysis. Thus, LPS with a short polysaccharide chain (LPS from mutant bacteria) is used to produce Lipid A products. The most extreme mutants are the Re mutants which produce an LPS which is made up of Lipid A and 3-deoxy-D-manno-octulosonic acid (2-keto-3-deoxyoctonate, KDO) as the sole constituent of the core.⁴ Lipid A and lipopolysaccharides from rough strains are tested for KDO content.⁵ The measure of the remaining KDO in the lipid A is a measure of the efficiency of hydrolysis. The preparation is considered pure if there is less than 0.2% KDO in the product. The lipopolysaccharides and the Lipid A products are all diphosphorylated (1, 4') unless noted as monophosphoryl (4').

Lipid A is the endotoxic principle of lipopolysaccharides. Free lipid A has been shown to exhibit most of the endotoxic reactions of the parent lipopolysaccharide; however, free lipid A did not induce necrosis and regression of tumors in mice.⁶ Lipid A is of great pathophysiological interest since it exerts many profound effects when injected into animals, including the induction of endotoxic shock,⁷ pyrogenicity,⁸ macrophage activation,⁹ B lymphocyte mitogenicity,¹⁰ induction of interferon production¹¹ complement activation,¹² and tumor regression.¹³

Monophosphoryl lipid A is nontoxic, whereas diphosphoryl lipid A is toxic.³ Monophosphoryl Lipid A has been reported to be used in the preparation of liposomes for antigenic studies.¹⁴ Monophosphoryl Lipid A is a component of the Ribi Adjuvant System.

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

Diphosphoryl Lipid A is miscible in chloroform:methanol:water (74:23:3) (10 mg/ml), yielding a slightly hazy, yellow solution. Diphosphoryl Lipid A is also soluble in 0.2% triethylamine (1 mg/ml) and in DMSO (1 mg/ml). Both solutions were faintly hazy and colorless after sonication. This product is not soluble in DMSO (10 mg/ml).

References

1. Qureshi, N., et al., Position of ester groups in the lipid A backbone of lipopolysaccharides obtained from *Salmonella typhimurium*. *J. Biol. Chem.*, **258(21)**, 12947-12951 (1983).
2. Chang, C. C. and Nowotny, A., Relation of structure to function in bacterial O-antigens--VII. Endotoxicity of "lipid A". *Immunochemistry*, **12(1)**, 19-28 (1975).

3. Qureshi, N., et al., Purification and structural determination of nontoxic lipid A obtained from the lipopolysaccharide of *Salmonella typhimurium*. J. Biol. Chem., **257(19)**, 11808-11815 (1982).
4. Raetz, C. R., Biochemistry of endotoxins. Annu. Rev. Biochem., **59**, 129-170 (1990).
5. Cynkin, M. A., and Ashwell, G., Estimation of 3-deoxy sugars by means of the manonaldehyde-thibarbituric acid reaction. Nature, **186**, 155-156 (1960).
6. Luderitz, O., et al., in Current topics in membranes and transport, vol. 17, Bronner, F., and Kleinzeller, A., eds., Academic Press (New York, NY: 1982), pp. 79-151.
7. Ribi, E. E., et al., Enhancement of endotoxic shock by N-acetylmuramyl-L-alanyl-(L-seryl)-D-isoglutamine (muramyl dipeptide). Cancer Res. **39(11)**, 4756-4759 (1979).
8. Galanos, C., et al., Biological activities of lipid A complexed with bovine-serum albumin. Eur. J. Biochem., **31(2)**, 230-233 (1972).
9. Weinberg, J. B., et al., Characterization of the effects of endotoxin on macrophage tumor cell killing. J. Immunol., **121(1)**, 72-80 (1978).
10. Chiller, J. M., et al., Relationship of the structure of bacterial lipopolysaccharides to its function in mitogenesis and adjuvanticity. Proc. Natl. Acad. Sci. U S A., **70(7)**, 2129-2133 (1973).
11. Schiller, J. G., et al., Interferon production in mice by components of *Salmonella minnesota* R595 lipid A. Infect. Immun., **14(2)**, 586-589 (1976).
12. Mergenhagen, S. E., et al., Activation of complement by endotoxin. J. Infect. Dis., **128** Suppl:86-90, (1973).
13. Ribi, E. E., et al., Tumor regression caused by endotoxins and mycobacterial fractions. J. Natl. Cancer Inst., **55(5)**, 1253-1257 (1975).
14. Richards, R. L., et al., Liposomes Containing Lipid A Serve as an Adjuvant for Induction of Antibody and Cytotoxic T-Cell Responses against RTS,S Malaria Antigen. Infect. Immun., **66(6)**, 2859-2865 (1998).

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