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CERTIFICATE OF ANALYSIS LIPOPOLYSACCHARIDE from *Escherichia coli* 0111:B4 Lot #20144A2

Contents:

Each vial contains 5.0 mg of lyophilized lipopolysaccharide (LPS) isolated from *Escherichia coli* 0111:B4 by a modification of the phenol extraction method of Westphal and Jann.¹

Packaging/Reconstitution/Storage:

LPS is dispersable in aqueous solvents at concentrations of 1.0 mg/ml. To achieve a suspension in water, heating to approximately 50°C with intermittent vortexing or sonication is generally recommended ². Allow ample time for dispersion to occur. The use of 0.5% triethylamine aids in dispersion. Triethylamine is very basic and may be neutralized with Tris HCl to avoid hydrolysis of the fatty acid chains. It is recommended that this material be stored at 2-8°C prior to and following reconstitution.

Endotoxin Activity:

The endotoxin activity, determined using a kinetic chromagenic LAL assay, is approximately 9.56×10^6 EU/mg.

Analysis:

2-Keto-3-deoxyoctonate (KDO) ³	. 2.8%
Phosphate ⁴	3.0%
Protein ⁵	1.47%
Nucleic acid ⁶	0.87%

Handling:

Good laboratory technique should be employed in the safe handling of any lipopolysaccharide or lipid A product. This requires observing the following practices:

 Wear appropriate laboratory attire including a lab coat, nitrile gloves and safety glasses. Nitrile gloves are recommended when handling lyophilized material.

(continued)

- 2. Do not mouth pipette, inhale, ingest or allow to come into contact with open wounds. Wash thoroughly any area of the body which comes into contact with the product.
- 3. This product is pyrogenic. Avoid accidental autoinoculation by exercising extreme care when handling in conjunction with any injection device.
- 4. This product is intended for research purposes by qualified personnel only. It is not intended for use in humans or as a diagnostic agent. List Biological Laboratories, Inc. is not liable for any damages resulting from the misuse or handling of this product.

FOR RESEARCH PURPOSES ONLY. NOT FOR USE IN HUMANS.

References:

- 1. Westphal, O. and Jann, K. (1965) Bacterial Lipopolysaccharides in *Methods* in *Carbohydrate Chemistry* 5, Whistler, R.L. ed., Academic Press, New York, pp. 83-91.
- 2. Mukerjee, P., Kastowsky, M., Obst, S., Takayama, K. (1999)
 Lipopolysaccharide Preparations in Aqueous Media in *Endotoxin in Health and Disease*, Brade, H., Opal, S.M., Vogel, S.N., Morrison, D.C. eds., Marcel Dekker, Inc., New York, p. 223-224.
- 3. Cynkin, M.A. and Ashwell, G. (1960) Nature 186, 155-156.
- 4. Ames, B.N. and Dubin, D.T. (1960) *J. Biol. Chem.* **235**, 769-775.
- 5. Bradford, M.M. (1976) Anal. Biochem. 72, 248-254.
- 6. Determined by absorption at 260 nm.