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62325 Lipopolysaccharide from Escherichia coli Serotype 0111:B4

Structure:
In their purest form, in the presence of strong surface active agents and in the absence of divalent cations, bacterial endotoxins consist of 10,000-20,000 dalton molecules made up of a lipid part (lipid A, which is responsible for the toxic properties of the molecule), a core polysaccharide and an O-antigenic polysaccharide side chain (specific to the bacterial serotype). Purified endotoxin is generally referred to as LPS, to distinguish it from its more natural protein complexed cell membrane associated form.
The only LPS from E. coli which exhibits short chain-length behavior on SDS-PAGE is LPS from serotype 026:B6. In fact, the short chain-length of this LPS is closer to that of the rough strains, which are mutants with short chain-length LPS's.
The following are useful definitions for bacterial classifications:

Genus:
Examples of genus are "Escherichia" in *Escherichia coli* and "Salmonella" in *Salmonella typhimurium*. The genus in the name of the organism is always capitalized.

Species:
Examples of species are "coli" in *E. coli* and "typhimurium" in *Salmonella typhimurium*. The species in the name of the organism is never capitalized.

Serotype:
Bacteria with common serotypes have surface antigens (e.g., group O, group H or LPS) which generate the same antibody response. Examples of serotypes are 055:B5 and 026:B6 for the *E. coli* bacterium. These designations are in fact immunological classifications which specify which antibody recognized which strains. Different strains may have some common antigenic determinants.

Strain, Variety or Subtype:
Different strains of the same bacterium differ in their requirements for sugars needed for growth (see PHENOTYPE and GENOTYPE definitions). Different sources of bacteria give different strains (e.g., *E. coli* from sheep feces will be of a different strain than *E. coli* from monkey feces). Examples of strains of *E. coli* are EH100 and F583.

Phenotype:
The phenotype designates how an organism behaves in culture. For example, it cannot grow with lactose as the nutrient sugar.

Genotype:
The genotype describes the genetic makeup of an organism (i.e., what genes is possesses and what it expresses). For example, it may contain the LacI and/or LacZ gene(s). If an organism does not posses a complete set of all 4 lac genes, it cannot use lactose as the nutrient sugar to grow in culture (see PHENOTYPE above).

Wild Type or Wild Strain:
A bacterium which occurs in nature and has not been deliberately genetically mutated in a laboratory.

Mutant or Rough Strain:
If a wild strain of bacterium is brought into a lab and irradiated with UV light or exposed to mutagenic compounds, it will mutate. Most mutations are lethal. The few which are not lethal result in viable mutants (a.k.a. rough strains) which are generally not found nature, and which possess some unique characteristics. The genes which encode lipopolysaccharide chains are formed. Ra, Rb, Rc, Rd, Rd, etc (where a, b, c, etc... designate 1^{st}, 2^{nd}, 3^{rd} , etc... degree, respectively) designate the polysaccharide length of a given LPS.

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Physicaly Properties:
Since the LPS is heterogeneous and tends to form different aggragates of varying size, the molecular weight is not very meaningful. However, there is a reported range of 1-4 million or greater. When the LPS is treated with SDS and heat, the molecular weight is in a range of 50,000 to 100,000.
References on studies of molecular structure are Jann et al., Eur. J. Biochem. Vol. 60, 239, 1975 and Methods in Enzymology, 28, 254.[1,2]

Applications:
Current Protocols in Immunology, Coligan et al. editors, John Wiley & Sons, NY, 1991 states that in Section 3.10 "Proliferative asays for B-cell function" that serotype 0111B4 from E. coli is recommended to activate B-cells.[3,5]
Serotype 0111:B4 is used to stimulate macrophages, per Geller, D.A. et al., PNAS USA, 90, 3491-3495 (1993).[4,5]
Morrison and Ryan used LPS for activating host cells to produce a spectrum of lymphokines and monokines including the interferons (alpha, beta, gramma), interleukins 1 and 6, tumor necrosis factor, platelet activating factor and procoagulant tissue factor.[6]
Further, it has been reported that LPS or the active lipid A component of it initiate a variety of biochemical pathways including protein kinase C,[7] cAMP dependent protein kinase,[8] phosphatidyl inositol turnover,[9] arachidonate metabolism,[10] protein myristolation[11] and activation of G-proteins.[12]

Product Solubility:
Lipoploysaccharides are molecules that form micelles in every solvent. This explains the hazy solutions observed in water and PBS. Organic solvents will not give clearer solutions. Methanol was found to give turbid suspensions with floaters, while water gives homogeneously hazy solution.
A more concentrated solution may be made, perhaps 20 mg/mL in saline with vortexing and warming to 70-80 °C. The solution will be hazy. Perhaps TCA-extracted prep (resulting in lower amounts of RNA) will work better than either phenol-extracted or the crude prep, although we have not tried this.

Solution Stability:
Solutions (1 mg/ml) in saline or tissue culture medium can be stored frozen in aliquots. Repeated freeze/thaw cycles are not recommended. The aliquots will remain stable frozen for 1-2 years.
LPS products in solution should be stable in phosphate buffered saline at 1 mg/mL at 0-4 °C for at least a month. Frozen aliquots are recommended for long-term storage. The solution should be stored in silanized containers, since LPS's bind to glass. If glass is used, vortex solutions at least 30 minutes to redissolve bound LPS's.
At very low concentrations (<0.1 mg/mL in water), LPS tends to stick to containers made of certain types of glass or plastic. For this reason, pre-heated soda glass vials are used to test the endotoxin acitivity of LPS. If the LPS concentration is >1 mg/mL, adsorption to the sides of the vial is negligible.

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