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SIGMA

ProductInformation

Lipopolysaccharides
from *Escherichia coli* K-235

Product Number **L 2268**
Storage Temperature 2-8 °C

Product Description
Synonym: LPS

This product is TCA extracted from *E. coli* serotype K235. The source strain is ATCC 13027.

Lipopolysaccharides (LPS) are characteristic components of the cell wall of Gram negative bacteria; they are not found in Gram positive bacteria. They are localized in the outer layer of the membrane and are, in noncapsulated strains, exposed on the cell surface. They contribute to the integrity of the outer membrane, and protect the cell against the action of bile salts and lipophilic antibiotics.[1]

Lipopolysaccharides are made up of a hydrophobic lipid (lipid A, which is responsible for the toxic properties of the molecule), a hydrophilic core polysaccharide chain, and a hydrophilic O-antigenic polysaccharide side chain. In most cases, O-specific chains are built of repeating units of oligosaccharides which exhibit a strain-specific structural diversity. The sugar constituents, their sequence, and their mode of linkage determine the serological O specificity of respective strains. They are the main determinants of the classifications of the serotypes of S*almonella* species. The diversity of O chains in *Enterobacteriaceae* may have developed during evolution to allow enteric bacterial to escape the host's immune system by developing new specificities on their cell surface (specific to the bacterial serotype).[1]

Since lipopolysaccharides confer antigenic properties on the cell, they have been termed O antigens. As the main antigen, lipopolysaccharides are involved in various host-parasite interactions. They seem to protect Gram negative bacteria from phagocytosis and lysis.[1] Bacteria with common serotypes have surface antigens (group O, group H, or LPS) which generate the same antibody response. Examples of serotypes are O55:B5 and O26:B6 for the *E. coli* bacterium. The designations are immunological classifications, which specify which antibody recognized which strains. Different strains may have some common antigenic determinants.

If a wild strain of bacterium is irradiated with UV light or exposed to mutagenic compounds, it will mutate. The few mutations that are not lethal result in viable mutants (rough strains) which are generally not found in nature, and which possess some unique characteristics. The genes that encode lipopolysaccharide formation may also be altered in the mutants, and LPS with shorter polysaccharide chains may be formed. Ra, Rb, Rc, Rd, Re, etc. (where a, b, c, etc... designate 1st, 2nd, 3rd, etc... degree, respectively) designate the polysaccharide length of a given LPS. Ra and Re designate the mutants with the longest and shortest chain lengths, respectively.[2] 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.[2] Lipid A and lipopolysaccharides from rough strains are tested for KDO content.[3]

Purified endotoxin is generally referred to as lipopolysaccharide or LPS, to distinguish it from the more natural complexed cell membrane associated form. The core portion of the polysaccharide chain is common to LPS from wild and mutant bacterial strains.

Removal by hydrolysis of the polysaccharide chain from LPS produces lipid A, either as the naturally occurring, cytotoxic diphosphoryl form[4] or the less toxic, monophosphoryl form.[5,6] The longer the polysaccharide chain is, the longer and more difficult the hydrolysis. LPS with a long polysaccharide chain has a relatively low lipid A content, which must be purified from a large amount of hydrolysis byproducts (oligosaccharides and saccharide monomers). Thus, the yield of lipid A is low and recovery is poor. LPS with a short polysaccharide chain (LPS from mutant bacteria) is therefore used to produce lipid A products. Removal of the fatty acid portions of lipid A results in a detoxified LPS[7] with an endotoxin level about 10,000 times lower than that of the parent LPS.

The molecular structure of LPS has been studied.[8,9] Since LPS is heterogeneous and tends to form aggregates of varying sizes, 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 the range of 50 to 100 kDa. In their purest form, in the presence of strong surface active agents, and in the absence of divalent cations, bacterial endotoxins consist of 10-20 kDa macromolecules. In the absence of surface active agents and in the presence of divalent cation sequestering agents such as EDTA, LPS is believed to arrange itself into a micellar structure with a molecular weight of approximately 1,000 kDa. This is the smallest form of bacterial LPS that is likely to exist in aqueous liquids. In the presence of divalent cations such as Ca^{2+} and Mg^{2+}, a bilayer structure appears to exist that passes through a 0.2 µm membrane, but does not pass through a 0.025 µm membrane. LPS vesicles up to 0.1 µm in diameter may also be formed in water in the presence of divalent cations. The self aggregation of LPS is generally a function of the lipid A component of the molecule, which also confers the ability to bind to hydrophobic surfaces.

LPS can be prepared by TCA,[10] phenol,[11] or phenol-chloroform-petroleum ether (for rough strains)[12] extraction. The TCA extracted lipopolysaccharides are structurally similar to the phenol extracted ones. Their electrophoretic pattern and endotoxicity are similar. The main differences are in the amounts of nucleic acid and protein contaminations. The TCA extract contains approximately 2% RNA and approximately 10% denatured proteins. The phenol extract contains up to 60% RNA and less than 1% protein. Purification by gel filtration chromatography removes much of protein present in the phenol-extracted LPS, but leaves a product that still contains 10-20% nucleic acids. Further purification using ion exchange chromatography, will yield an LPS product which contains <1% protein and <1% RNA. Sigma offers LPS with various levels of protein and/or RNA.

Sigma's lipopolysaccharides contain endotoxin levels of not less than 500,000 EU (endotoxin units)/mg unless otherwise noted. One nanogram of endotoxin is equivalent to 5 EU (Limulus lysate assay) and 10 EU (chromogenic assay).

LPS preparations are used extensively for research in the elucidation of LPS structure,[13] metabolism,[14] immunology,[15] physiology,[16] toxicity,[17] and biosynthesis.[18] They have also been used to induce synthesis and secretion of growth promoting factors such as interleukins.[19]

FITC (fluorescein isothiocyanate), TRITC (tetramethyrhodamine isothiocyanate), and TNP (trinitrophenyl) conjugates have been prepared by reacting LPS with either FITC, TRITC or 2,4,6-trinitrobenzenesulfonic acid, respectively.[20] They are used in research on the T-independent B cell immune response to bacterial LPS.[20]

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

Preparation Instructions
The product is soluble in water (5 mg/ml) or cell culture medium (1 mg/ml) yielding a hazy, faint yellow solution. A more concentrated, though still hazy, solution (20 mg/ml) has been achieved in aqueous saline after vortexing and warming to 70-80 °C.[21] Lipopolysaccharides are molecules that form micelles in every solvent. Hazy solutions are observed in water and phosphate buffered saline. Organic solvents do not give clearer solutions. Methanol yields a turbid suspension with floaters, while water yields a homogeneously hazy solution.

For cell culture use, LPS should be reconstituted by adding 1 ml of sterile balanced salt solution or cell culture medium to a vial (1 mg) and swirling gently until the powder dissolves. Solutions can be further diluted to the desired working concentration with additional sterile balanced salt solutions or cell culture media.

Storage/Stability
Solutions at 1 mg/ml in buffer or culture medium are stable for approximately one month at 2-8 °C. Frozen aliquots can be stored up to 2 years. Repeated freeze/thaw cycles are not recommended. Solutions should be stored in silanized containers, since LPS can bind to plastics and certain types of glass (especially at concentrations of <0.1 mg/ml). If the LPS concentration is >1 mg/ml, adsorption to the sides of the vial is negligible. If glass containers are used, solutions should be vortexed for at least 30 minutes to redissolve the adsorbed product.

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21. Customer report

LPS Table

Source organism	Extraction method	Gel Filtration	Gel Filtration γ-irr.	Ion-exchange	Detoxified	Gel Filtration FITC label	Gel Filtration TNP label
O26:B6 *E. coli*	Phenol - L8274 TCA – L3755	L2762	L2654			F7037*	T7143*
O55:B5 *E. coli*	Phenol - L2880 TCA – L4005	L2637	L6529	L4524	L9023	F8666	T6682
O111:B4 *E. coli*	Phenol – L2630 TCA – L4130	L3012	L4391	L3024	L3023	F3665	T3382
O127:B8 *E. coli*	Phenol – L3129 TCA – L3880	L3137	L4516	L5024	L8654	F3540*	
O128:B12 *E. coli*	Phenol – L2755 TCA – L4255	L2887					T6769
E. coli EH-100 (Ra mutant)	Ph/Chl/Pet - L9641						
E. coli F-583 (Rd mutant)	Ph/Chl/Pet – L6893						
E. coli J5 (Rc mutant)	Ph/Chl/Pet – L5014		L7520*				
E. coli K-235	Phenol – L2143 TCA – L2268	L2018					
Klebsiella pneumoniae	Phenol – L4268 TCA – L1519	L1770*					
Pseudomonas aeroginosa 10	Phenol – L9143 TCA – L7018	L8643					
Salmonella abortus equi	Phenol - L5886 TCA – L6636	L1887					
Salmonella enteritidis	Phenol - L6011 TCA – L6761	L2012	L7770	L4774	L3773		
Salmonella minnesota	Phenol - L6261 TCA – L7011	L2137	L4641		L1523*	F4665*	T3520*
Salmonella minnesota strain R5	Ph/Chl/Pet - L8893						
Salmonella minnesota strain R7 (Rd mutant)	Ph/Chl/Pet - L9391						
Salmonella minnesota strain Re595 (Re mutant)	Ph/Chl/Pet - L9764		L7645*				
Salmonella typhimurium	Phenol – L6511 TCA - L7261	L2262	L6143		L2525	F4790*	T4145*
Salmonella typhimurium strain SL684 (Rc mutant)	Ph/Chl/Pet - L5891						
Salmonella typhimurium strain SL1181 (Re mutant)	Ph/Chl/Pet - L9516						
Salmonella typhimurium strain TV119 (Ra mutant)	Ph/Chl/Pet - L6016						
Salmonella typhosa	Phenol - L6386 TCA - L7136	L2387	L7895			F4292*	
Serratia marcescens	Phenol - L6136	L2512*	L4766*				
Shigella Flexneri A1	Phenol – L4393 TCA – L7143	L9018					
Shigella flexneri (Re mutant)	Ph/Chl/Pet - L6643						
Vibrio cholerae serotype Inaba 569B	Phenol - L0385					F5009*	T1271*

* = discontinued product number
Ph/Chl/Pet = phenol:chloroform:petroleum ether