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Fluorescent Conjugates of Lipopolysaccharides (LPS)

Quick Facts

Storage upon receipt:

- –20°C
- Desiccate
- Protect from light

Note: Pyrogenic; handle with care.

Table 1. *Fluorescent lipopolysaccharide conjugates.*

Label (Abs/Em) *	*Escherichia coli* Serotype 055:B5	*Salmonella minnesota*
BODIPY® FL (503/513) †	L-23350	L-23355
Alexa Fluor® 488 (495/519)	L-23351	L-23356
Alexa Fluor® 568 (578/603)	L-23352	L-23357
Alexa Fluor® 594 (590/617)	L-23353	L-23358

* Absorbance and fluorescence emission maxima in nm. † At high concentrations, such as in micelles or aggregates, BODIPY FL emission maximum will shift from ~513 nm to ~620 nm.[11,12]

Introduction

Lipopolysaccharides (LPS) or endotoxins are complex macromolecules present on the outer cell walls of gram negative bacteria. The structural core of LPS, and the primary determinant of its biological activity, is the *N*-acetylglucosamine derivative, lipid A (Figure 1). If the fatty acid residues are removed from the lipid A component, the toxicity of the LPS can be reduced significantly. However, the mono- or diphosphoryl forms of lipid A are inherently toxic.[1] In many gram-negative bacterial infections, endotoxins are responsible for clinically significant symptoms like fever, low blood pressure and tissue edema, which can lead to disseminated intravascular coagulation, organ failure and death.[1] Studies also clearly indicate that endotoxins are responsible for various signal transduction pathways including those involving protein kinase C [2,3] and protein myristylation [4] as well as for stimulating a variety of immunochemical responses including B lymphocyte [5] and G-protein activation.[6]

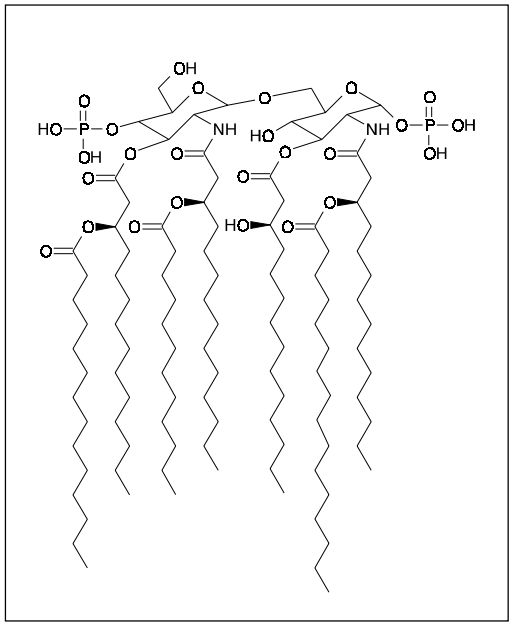

Figure 1. *Structure of the lipid A component of Salmonella minnesota lipopolysaccharide.*

Molecular Probes offers several fluorescent conjugates of LPS from *Escherichia coli* and *Salmonella minnesota* (Table 1). Fluorescent conjugates of LPS will help researchers to follow LPS binding and transport processes. Cell internalization studies can utilize trypan blue to quench the fluorescence of surface bound LPS.[7] In one study a BODIPY® FL derivative of LPS from *E. coli* strain LCD25 was used to measure the transfer rate of LPS from monocytes to high-density lipoprotein (HDL).[7] Another study utilized a BODIPY FL derivative of LPS from *S. minnesota* to demonstrate transport to the Golgi apparatus in neutrophils,[8,9] although this could have been due to probe metabolism. Molecular Probes' fluorescent LPS conjugates can potentially be combined with other fluorescent indicators, such as calcium-, pH- or organelle-specific stains to monitor intracellular localization as well as real-time changes in cellular response to LPS.

Storage and Handling

The lipopolysaccharide conjugates are supplied lyophilized in unit sizes of 100 µg. They should be stored desiccated at -20°C. If organic solvents are used to dissolve the conjugates, the reconstituted solutions should be transferred to glass vials and stored desiccated at –20°C. If aqueous solvents are used, the solution should be divided into aliquots and stored at –80°C. PROTECT SOLUTIONS FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

Caution: These products are potentially pyrogenic. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses. Do not mouth pipette, inhale, ingest or allow to come into contact with open wounds. Wash thoroughly any area of the body that comes into contact with this product.

- ***Escherichia coli* LPS:** The molecular weight of *E. coli* Serotype O55:B5 LPS is approximately 10,000 daltons. Solutions of 1–10 mg/mL can be made by dissolving the powder in the appropriate amount of phosphate-buffered saline (PBS) or other suitable buffer. Heating to 37°C and vortexing or sonicating may be required.
- ***Salmonella minnesota* LPS:** The molecular weight of *S. minnesota* LPS is approximately 3000 daltons. Solutions of 1–10 mg/mL can be made by dissolving the powder in the appropriate amount of phosphate-buffered saline (PBS) or other suitable buffer. Heating to 37°C and vortexing or sonicating may be required.

Applications

LPS forms micelles in aqueous buffers. Micelles or aggregates of LPS bind poorly and induce a response only at very high concentrations. Liposomes created with LipofectAMINE™ containing these aggregates have been shown to be more efficiently internalized via endocytosis.[13]

Materials That May Be Required, but Not Supplied:

- LipofectAMINE™, available from Life Technologies

Protocol

1. Prepare a 10 µg/mL solution of the fluorescent LPS conjugate in complete medium containing LipofectAMINE at a final concentration of 6 µg/mL.

2. Prepare liposomes by agitating this solution on a vortex mixer.

3. Rinse cells in an appropriate medium (such as HBSS or HEPES).

4. Prewarm the medium containing the liposomes to 37°C. Incubate the cells for 1 hour at 37°C with this solution.

5. Wash the sample in an appropriate medium and examine using a fluorescence microscope equipped with an appropriate filter set for the fluorophore.

Alternatively the addition of plasma dramatically enhances the ability of LPS to both bind to cells and evoke responses.[10] Two plasma proteins, LPS-binding protein (LBP) and soluble CD14 (sCD14), have been shown to play a large role in transporting LPS and in mediating responses of cells [8,9,11] Protocols for delivery of monomeric LPS should be obtained from the primary literature.[8,9,11]

References

1. Chem Immunol 74, 5 (2000); **2.** J Exp Med 183 1899, (1996); **3.** J Biol Chem 259, 10048 (1984); **4.** Proc Natl Acad Sci USA 83, 5817 (1986); **5.** Adv Immunol 28, 293 (1979); **6.** Eur J Immunol 19, 125 (1989); **7.** J Biol Chem 274, 34116 (1999); **8.** J Exp Med 190, 509 (1999); **9.** J Exp Med 190, 523 (1999); **10.** J Immunol 155, 6 (1995); **11.** J Biol Chem 271, 4100 (1996); **12.** J Immunol 158, 3925 (1997); **13.** BioTechniques 28, 510 (2000).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
L-23351	lipopolysaccharides from *Escherichia coli* serotype 055:B5, Alexa Fluor® 488 conjugate	100 µg
L-23352	lipopolysaccharides from *Escherichia coli* serotype 055:B5, Alexa Fluor® 568conjugate	100 µg
L-23353	lipopolysaccharides from *Escherichia coli* serotype 055:B5, Alexa Fluor® 594 conjugate	100 µg
L-23350	lipopolysaccharides from *Escherichia coli* serotype 055:B5, BODIPY® FL conjugate	100 µg
L-23356	lipopolysaccharides from *Salmonella minnesota*, Alexa Fluor® 488 conjugate	100 µg
L-23357	lipopolysaccharides from *Salmonella minnesota*, Alexa Fluor® 568 conjugate	100 µg
L-23358	lipopolysaccharides from *Salmonella minnesota*, Alexa Fluor® 594 conjugate	100 µg
L-23355	lipopolysaccharides from *Salmonella minnesota*, BODIPY® FL conjugate	100 µg

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