ALPHA DIAGNOSTIC INTERNATIONAL

Product Data Sheet

□ Cat # RP-829 **Recombinant Listeriolysin-O PEST-free** **Size**: □ 10 ug

Listeriolysin O (aka LLO) is a hemolysin produced by Listeria monocytogenes bacteria, the pathogen responsible for causing listeriosis. The toxin may be regarded as a virulence factor, since it is crucial for the virulence of L. monocytogenes. LLO is a single polypeptide protein encoded by the *hlyA* gene and composed of 529 residues. LLO is a thiol-activated cholesterol-dependent pore forming toxin protein; therefore, it is activated by reducing agents and inhibited by oxidizing agents. Still, LLO differs from other thiol-activated toxins, as its cytolytic activity is maximized at a pH of 5.5. Inside the acidic phagosomes (average pH ~ 5.9) of cells that have phagocytosed L. monocytogenes, LLO is selectively activated by maximizing activity at a pH of 5.5. Following the phagosome lysis by LLO, the bacterium breaks out into the cytosol, where it is able to grow intracellularly, and the toxin has reduced activity in the more basic cytosol. Thus, LLO permits L. monocytogenes to break out from the phagosomes into the cytosol without harming the plasma membrane of the infected cell, which allows the bacteria to live intracellularly, where they are sheltered from extracellular immune system factors such as the complement system and antibodies. LLO also brings about dephosphorylation of histone H3 and deacetylation of histone H4 in the early phases of infection, before entry of L. monocytogenes into the host cell. The pore-forming activity is not implicated in causing the histone modifications. The modifications of the histones affect the down regulation of genes encoding proteins involved in the inflammatory response. Therefore, LLO may be significant in subverting the host immune response to L. monocytogenes. At its NH_2-terminus it possesses a 25 residues long typical signal sequence excited during the secretion process. Moreover, in its NH_2-terminus there is also a 19 amino acids PEST- like sequence that may target this toxin for degradation. The PEST-like sequence found in LLO and is considered crucial for virulence, given that mutants lacking the sequence lysed the host cell. Nevertheless, contrary to PEST's supposed role in protein degradation, evidence implies that the PEST-like sequence may control LLO production in the cytosol rather than increase degradation of LLO.

Background:

Listeriolysin O (LLO) is a single polypeptide protein secreted by the Gram-positive bacterium Listeria monocytogenes. LLO belongs to the group of cholesterol-binding sulfhydrylactivated toxins, the lytic activity of which is enhanced by reducing agents and is suppressed by exposure to oxygen or cholesterol. LLO hemolytic activity is maximum at pH 5.5 and rapidly decreases with the increase of the pH.

Recombinant Listeriolysin O lacking both the signal secretion sequence and the PEST- like sequence. LLO-PEST minus is composed of 471 amino acids, starting from amino acid 60 to amino acid 529, with the addition of a methionine in its NH2-terminus.

Source:

rLLO Expressed in Escherichia Coli and purified (>95%). The protein (0.357mg/ml) contains 50mM NaH2PO4, 1mM EDTA, 2.7mM KCl, 1mM DTT, 5% glycerol and 0.5M NaCl.

Stability:
rLLO although stable at room temperature for 1 week, should be stored desiccated below -18°C. Please prevent freeze-thaw cycles.

Activity :

rLLO has a hemolytic activity 1.25x106 HU/mg. 2mM DTT is used to reactivate the toxin.

Applications:
a. Cytosolic delivery of molecules, peptides, oligonucleodides and plasmid DNA (4). b. Production of specific monoclonal antibodies (5). c. Detection of anti-listeriolysin O antibodies (6).

References: 1. Rocourt, J., and Cossart, P. (1997) Listeria monocytogenes in "Food microbiology: fundamentals and frontiers" (Doyle, M. P., Beuchat, L. R., and Montville, T. J., Ed.), pp. 337-352, ASM Press, Washington D.C.; 2. Palmer, M. (2001) The family of thiol-activated, cholesterol-binding cytolysins. Toxicon 39, 1681-1689.
3. Giammarini C., Andreoni F., Amagliani G., Casiere A., Barocci S. and Magnani M. (2003) High-level expression of the Listeria monocytogenes listeriolysin O in Escherichia coli and preliminary characterization of the purified protein. Protein Expr. Purif. 28: 78-85; 4. Provoda C. J. and Lee K.-D. (2000) Bacterial poreforming hemolysins and their use in the cytosolic delivery of macromolecules. Adv. Drug. Deliv. Rev. 41: 209-221; 5. Nato F., Reich K., Lhopital S., Rouyre S., Geoffroy C., Mazie J. C. and Cossart P. (1991) Production and characterization of neutralizing and nonneutralizing monoclonal antibodies against listeriolysin O. Infect. Immun. 59: 4641- 4646; 6. Barbuddhe S. B., Malik S. V. S. and Gupta L. K. (2000) Kinetics of antibody production and clinical profiles of calves experimentally infected with Listeria monocytogenes. J. Vet. Med. 47: 497-502

Usage:

This item is for LABORATORY ESEARCH USE ONLY. The product may not be used as drugs, agricultural or pesticidal products, food additives or household chemicals If supplied in powder then reconstitute it in 100 ul water for 1 mg/ml stock and store in liquid at 4oC for ~1 week or aliquots in suitable size and store at -20oC for long term storage..

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