RayBiotech, Inc.

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Certificate of Analysis and Data Sheet

Recombinant Bacterial Outer Membrane Protein-A

Catalog No.	**Source:**
228-11173	*Escherichia coli*

Synonyms

Outer Membrane Protein-A, OmpA.

Introduction

The OmpA protein is one of the main outer-membrane proteins of a large array of Gram-negative bacteria such as A. salmonicida, Shigella dysenteriae and E. coli. OmpA's major physiological functions include maintenance of the structural integrity and morphology of the cells and porin activity, as well as a role in conjugation and bacteriophage binding. Achromogenic atypical Aeromonas salmonicida is the causative agent of goldfish ulcer disease. Virulence of this bacterium is associated with the production of a paracrystalline outer membrane A-layer protein. The species specific structural gene for the monomeric form of A-protein was cloned into a pET-3d plasmid in order to express and produce a recombinant form of the protein in E. coli BL21(DE3). The induced protein was isolated from inclusion bodies by a simple solubilization-renaturation procedure and purified by ion exchange chromatography on Q-Sepharose to over 95% pure monomeric protein. Recombinant A-protein was compared by biochemical, immunological and molecular methods with the A-protein isolated from atypical A. salmonicida bacterial cells by the glycine and the membrane extraction methods.

Description

The recombinant form was found to be undistinguishable from the wild type when examined by SDS-PAGE and gel filtration chromatography yielding a 48 kDa monomeric protein. The immunological similarity of the protein samples was demonstrated by employing polyclonal and monoclonal antibodies in ELISA and Western Blot techniques. All forms of A-protein were found to activate the secretion of tumour necrosis factor alpha from murine macrophage. For ref see Maurice et al. (1999) Protein Expression and Purification 16, 396-404.
The OmpA is purified by proprietary chromatographic techniques.

Physical Appearance

Sterile Filtered White lyophilized (freeze-dried) powder.

Solubility

It is recommended to reconstitute the lyophilized OmpA in sterile 0.4% NaHCO3.

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Stability

Lyophilized Bacterial Outer Membrane Protein-A although stable at room temperature for 3 weeks, should be stored desiccated below -18°C. Upon C between 2-7 days and for future use reconstitution OmpA should be stored at 4 below -18°C. For long term storage it is recommended to add a carrier protein (0.1% HSA or BSA).
Please prevent freeze-thaw cycles.

Purity

Greater than 98.0% as determined by
(a) Analysis by RP-HPLC.
(b) Analysis by SDS-PAGE.

Amino acid sequence

The sequence of the first five N-terminal amino acids was determined and was found to be Met-Asp-Val-Val-Ile-Ser.

Biological Activity

The interaction of bacterial and recombinant A-layer protein with murine macrophages was directed at determining the effect of A-protein on intracellular events that occur in primed macrophages. This was accomplished by measuring the cytotoxic product produced by peritoneal macrophages when exposed to A-protein coated latex beads. Thioglycolate elicited macrophages exhibited a low level of activation (18% cytotoxicity) that was significantly increased (48% cytotoxicity) in the presence of latex beads. Coating of the latex beads with each of the three A-protein products resulted in an increase of cytoxicity (mean +/- SEM) from 48% to 91%.