



Product Datasheet

Product Name	Outer Membrane Protein-A Bacterial Recombinant
Cata No	CB501182
Source	<i>Escherichia Coli.</i>
Synonyms	Outer Membrane Protein-A, OmpA.

Description

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.

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.

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 cytotoxicity (mean +/- SEM) from 48% to 91%.

Purity

Greater than 98.0% as determined by:

- Analysis by RP-HPLC.
- Analysis by SDS-PAGE.

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Reconstitution

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

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 reconstitute the protein (0.1% HSA or BSA).

Please avoid freeze-thaw cycles.

Sequence

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

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