

E. coli Outer Membrane -A / ompA - Purified

Catalog No.: AR10506PU-L

Quantity: 1 mg

Background: 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.

Species: *E. coli*

Source: *E. coli*

Format: **State:** Sterile Filtered White lyophilized (freeze-dried) powder
Purity: >98.0% as determined by both RP-HPLC and SDS-PAGE analysis.
Purification Method: Proprietary chromatographic techniques.
Reconstitution: Restore in sterile 0.4% NaHCO₃

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.

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

Storage:

Prior to reconstitution store at 2-8°C for one month or desiccated below -18°C.

Following reconstitution store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.

For long term storage it is recommended to add a carrier protein (0.1% HSA or BSA).

Avoid repeated freezing and thawing.

Shelf life: one year from despatch.