Abstract

Thesis Title: Production of Polyclonal Antibodies Against Recombinant Protein V-ATPase

Subunit G of Hevea brasiliensis.

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V-ATPase is an enzyme membrane bounded involved in active transport. The objective of this study was to produce polyclonal antibodies against recombinant V-ATPase G subunit protein of Hevea brasiliensis. The V-ATPase G subunit gene was ligased into pET100/D-TOPO vector. Recombinant V-ATPase G subunit DNA was transformed into Escherichia coli BL21 StarTM(DE3) One Short® and optimized condition for induction of target protein. The optimal expressed condition are 0.5 mM IPTG at 37 °C for 3 hours. The recombinant V-ATPase G subunit protein was separated by SDS-PAGE and eluted target protein from polyacrylamide gel. The purified recombinant V-ATPase G subunit protein and Freund's Adjuvant were mixed and injected to immunize New Zealand White rabbit. Polyclonal antibodies from rabbit serum against recombinant V-ATPase G subunit protein and specificity test of these Polyclonal antibodies against V-ATPase G subunit protein was determine by Western Blot. These Polyclonal antibodies were also specific to V-ATPase G subunit protein from leaf and latex of Hevea Brasiliensis. using the same technique which resulted in the specific band.