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Antibody-based detection of alkaline phosphatase in lepidopteran insects (Lepidoptera: Noctuidae)

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ABSTRACT. Alkaline phosphatase (ALP) of *Helicoverpa armigera* Hub. (Lepidoptera; Noctuidae) (GenBank accession No. EU729322) was cloned and expressed. The target gene *H.a-ALP*, having an open reading frame of 1608 bp, was reverse-transcribed from cDNA by the polymerase chain reaction. The open reading frame of the target gene was cloned into the pET-32a expression vector to obtain recombinant protein in *Escherichia coli* DE-3 cells for the subsequent production of polyclonal antibody. New Zealand white rabbits were used for production of anti-pET-32a-H.a-ALP. The production of antibody was also optimized by employing ELISA for titer determination. The produced antiserum was processed and used as an antibody. Western blot results showed that the polyclonal antibody produced was capable of effectively binding target protein not only from *H. armigera* but also from other lepidopterans such as *Mythimna separata* and *Plutella xylostella*. This antibody was also used to detect levels of ALP within different instars of *H. armigera*. Thus, it is concluded that this antibody-based assay is very useful for the effective detection of gene-specific expression. Furthermore, it may also be used to detect the expression levels and tissue localization of ALP, as well as in

other physiological studies involving this enzyme.

Key words: Alkaline phosphatase; pET-32a expression vector; ELISA; *Helicoverpa armigera* Hub.; Polyclonal antibody

