

Puttarakun Meesiri 2015: Gene Cloning and Expression of Hepatitis B Surface Antigen in Mammalian cell line. Master of Science (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Associate Professor Captain Chaivat Kittigul, M.Sc. 83 pages.

Middle protein and small protein of hepatitis B surface antigen (HBsAg) were produced by gene cloning and expression in mammalian cell line. PCR amplification of *PreS2+S* and *S* genes from plasmids containing HBsAg genes were performed using primers with have secretory signal sequence and gave DNA 984 bp and 819 bp, respectively. Middle protein HBsAg DNA (984 bp) and small protein HBsAg DNA (819 bp) were inserted into the expression vector (pcDNA3.4) recombinant plasmid of pcDNA3.4/*PreS2+S* and pcDNA3.4/*S* were obtained, then, the recombinant plasmids were transfected into mammalian cell line and tested for HBsAg expression by enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA). The middle protein could be detected intracellular and in cell culture fluid 24 hours earlier than the small protein at 48 hours after transfection. The level of HBsAg expression increased until 72 hours after transfection. Western blot analysis of HBsAg reacted with anti-HBsAg polyclonal antibody showed specificity to the middle protein. This HBsAg may be useful for the development of diagnostic test and vaccine development of hepatitis B.

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Thesis Advisor's signature

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