

Kunan Bangphoomi 2007: Production of Human Monoclonal Antibody against CD3 ϵ of Domestic Duck *Anas Platyrhynchos* by Antibody Phage Display Library. Master of Science (Veterinary Pathology), Major Field: Veterinary Pathology, Department of Pathology. Thesis Advisor: Associate Professor Thaweesak Songserm, Ph.D. 125 pages.

Monoclonal and polyclonal antibodies against duck CD3 ϵ are useful as a cellular marker for differentiating T lymphocytes from B lymphocytes. The antibodies may be used for localization and enumeration of duck T lymphocytes in various preparations. Nevertheless, the antibodies are not commercially available. The objective of the present study was to produce polyclonal and monoclonal antibodies against the duck CD3 ϵ . DNA encoded ectodomain of duck CD3 ϵ was PCR amplified by using specific primers designed from the DNA sequence of *Anas platyrhynchos* (khaki Campbell duck) deposited in the GenBank and cDNA prepared from peripheral blood mononuclear cells (PBMC) of domestic duck as a template. The amplicon was cloned into pTrcHis2A expression vector and the recombinant vectors were introduced into DH5 α *E. coli*. The recombinant protein was produced and purified from a selected transformed DH5 α *E. coli* clone. The protein was used as an antigen in a phage biopanning to select phage clones displaying single chain antibody fragments (ScFv) specific to the ectodomain of CD3 ϵ chain from an established human antibody phage display library. The soluble ScFv was produced and purified from the phagemid transformed HB2151 *E. coli* clone. The recombinant protein was also used for mouse immunization to produce polyclonal antibodies. Western blot analysis revealed that both monoclonal and polyclonal antibodies reacted specifically to linear epitope of both recombinant CD3 ϵ protein and the native CD3 ϵ prepared from duck PBMC lysate. The CD3 ϵ specific antibodies have high potential as a cellular T cell marker for various research and diagnostic works.

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Thesis Advisor's signature

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