CHAPTER V

CONCLUSION

Antibody is generally a powerful tool in protein analyses, regarding to their indispensable biological properties. However, one of the most crucial steps in producing antibodies is the acquisition of highly purified immunogen which determine the opportunity to acquire the quality antibodies. In this study, a novel strategy in purifying and preparing a protein antigen for immunization purpose was introduced. The DNA fragment encoding the external domain of CD147 (CD147Ex) was cloned into pAK400CB plasmid vector which is composed of the C-terminal half of the biotin carboxyl carrier protein (BCCP) downstream to the cloning site. The BCCP domain is a natural substrate of biotin ligase that catalyse the conjugation of the biotin moiety to a specific lysine residue in the BCCP, leading to the spontaneous biotinylation of the fusion protein within the *E. coli* cells. The newly synthesized plasmid, named pAK400CB-CD147Ex, was transformed into *E. coli* Origami B, whose the oxidizing cytoplasmic environment promotes folding of the recombinant molecule. The CD147Ex-BCCP fusion protein was efficiently produced with adequate *in vivo* biotinylation after culturing at 25^{o}C with IPTG induction. As determined by ELISA, the strong reactivity of solid phase-immobilized CD147Ex-BCCP against six CD147 mAbs indicated the proper folding, the existence of CD147 bioactive epitopes, and biotin attachment of the fusion protein. The correct molecular size of the fusion molecule (30 kDa: CD147, 20 kDa plus BCCP, 9 kDa) was

demonstrated by CD147 mAbs and HRP-conjugated streptavidin in Western immunoblotting, confirming the presence of the CD147Ex-BCCP fusion protein with biotin attached.

The biotinylated CD147Ex-BCCP was subsequently sorted from the bacterial crude extract by being trapped on the streptavidin-coated magnetic beads, which were then separated from the solution mixture by the external magnetic field. This one-step method needs less time and cost consuming in comparison to other expression systems which require sophisticated purification steps (i.e. affinity chromatography). In order to produce antibody responses to CD147, the CD147Ex-BCCP-coated beads were used as immunogen. High titer of anti-CD147 mice polyclonal antibodies was observed by indirect ELISA. The specific antibody activity was still detected in the sera even at 5 months after the last immunization. The obtained mice antibodies were also demonstrated to strongly react against native membrane-bound CD147 by immunofluorescence using stable CD147-expressing BW5147 cells as target for staining. The positive fluorescent signal was further proven to be derived from the binding of mouse antibodies to the cell surface CD147 by Western immunoblotting in which the CD147-expressing BW5147 cell lysate was separated by SDS-PAGE, blotted to PVDF membrane, and subjected to react with mouse antibodies. The efficient antibodies activity obtained by this newly developed method demonstrated the excellence of our strategy in inducing the highly reactive and specific mouse polyclonal antibodies. This alternative immunogen preparation approach circumvented the cumbersome acquisition of purified immunogen in which the complicated purification processes are substituted with a single capturing step. Any target domains could be selectively cloned and expressed with *in vivo* biotinylation.

The magnetic beads anchoring target molecules via biotin-streptavidin interaction serve as particulate carrier for presenting the foreign molecules to the immune system. Thus, the biotinylated antigen-coated streptavidin magnetic beads represent the powerful immunizing agent and could be useful in challenging poorly immunogenic target proteins.