Production and purification of polyclonal antibody against mouse purified IgG2a subclass towards self-sufficiency of the country.

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Key word: purification, polyclonal antibody, IgG2a, ion-exchange chromatography, affinity chromatography

Background: Polyclonal antibodies are utilized in many fields of biomedical, biochemical and biological researches. Their ability to react with many epitopes of an antigen not only promotes their use in preparation of many immunoassay methods, but also makes them valuable reagents in research and diagnostic.

Methods: In this study proG and ProA affinity chromatography were carried out for purification of mouse IgG and IgG2a respectively. Verification method of the purified fractions was SDS-PAGE. Rabbit was immunized with purified IgG2a .After the final immunization, production of antibody was investigated by ELISA method. Rabbit serum was collected and precipitated at the final concentration of 50% ammonium sulfate and purified by ion-exchange chromatography and labeled with HRP. The titer and cross reactivity of HRP conjugated IgG was detected by direct ELISA method.

Results: The results of SDS-PAGE in reduced condition for determining the purity of mouse and rabbit IgG showed distinct band with molecular weight about 50-KDa at heavy chain MW position and the bands between molecular weights of 25-30 KDa at light chain MW position. In non-reduced condition, only one band was seen in about 150 KDa MW position. The titer of Rabbit Anti-mouse polyclonal antibody was 200000. The optimum titer of prepared HRP conjugated IgG was found about 4000. Analysis of cross reactivity with mouse IgG1, IgG2a, IgG3 showed that conjugated rabbit IgG has no cross reaction at optimized dilution.

Conclusion: Due to obtained high purity of mouse IgG2a subclass and rabbit polyclonal IgG, we concluded that affinity chromatography and ion-exchange chromatography could be appropriate techniques for purification of mouse IgG subclasses and rabbit IgG respectively. **Purified mouse IgG2a and HRP-conjugated IgG take more steps towards self-sufficiency of the country.**