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Preparation of anti-human IgG-hrp conjugate and its use in ELISA and western blotting experiments

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Conjugation of enzymes to antibodies involves the formation of a stable, covalent linkage between an enzyme [e.g., horseradish peroxidase (HRPO), urease, or alkaline phosphatase] and an antigen-specific monoclonal or polyclonal antibody in which neither the antigen-combining site nor the active site of the enzyme is functionally altered. The chemistry of cross-linking HRPO or urease to immunoaffinity purified monoclonal or polyclonal antibodies (IgG). The chemistry of cross-linking alkaline phosphatase to antibodies is presented. The enzyme most commonly used in the immunoreagent (the antibody enzyme conjugate) preparation is horseradish peroxidase. This enzyme is cheap and can be attached to the immunoreagent by a variety of methods. Moreover many chromogenic substrates for it are also available.

Critical parameters: The most critical parameters of both conjugation methods are the quality of enzyme and the cross-linking reagents. These reagents should be tested as described in the protocol before conjugating to larger quantities of antibodies. It is imperative that the *m*-maleimidobenzoyl N-hydroxysuccinimide ester (MBS), sodium periodate (NaIO_4) and sodium borohydride (NaBH_4) be stored in a dessicator and that solutions containing these chemicals be prepared immediately prior to use. The method described is applicable to most antibodies and should produce conjugates that are useful for developing an ELISA for detecting sensitively and specifically for a given antigen. However, not all antibodies conjugate in an identical manner. It may be necessary to vary the ratio of MBS/antibody or urease/antibody for the urease conjugation and the NaIO_4 /HRPO and HRPO/antibody ratios for a given HRPO conjugation. The quality and grade of alkaline phosphatase is crucial to the generation of effective conjugates. Immunoassay grade material is recommended over lower grades, and the enzyme should not be conjugated beyond its expiration date. In the case of polyclonal antisera, the specificity and titer of the antiserum will be reflected in the conjugate and any purification procedures that increase these values, such as immunoaffinity chromatography will enhance conjugate performance. The selection of an optimal conjugation time for preparing alkaline phosphatase–antibody conjugates varies for different antibodies, in particular when monoclonal antibodies are used. In contrast, polyclonal antibodies may be reliably conjugated in 120 min.

Worked in two parts:

Part-I: Preparations of anti human IgG -HRP conjugate

Part-II: Characterization of isolated human IgG

Biography

K Ramesh Kumar has completed M.Sc. Biochemistry from Osmania University. He has done project in preparation of anti human IgG-HRP conjugate and its use in ELISA and western blot. Participated in the poster presentation and quiz at the two day national seminar on "Emerging trends in science." Organized by NIN Hyderabad.

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