Immunoblot Detection of Antigens in Immunoprecipitates

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Co-immunoprecipitation of antigens from cell or tissue extracts is used in a wide variety of experimental systems to corroborate the existence of authentic protein-protein interactions in vivo. Typically, stable protein-protein associations are detected by immunoprecipitation with antibodies specific for one protein, followed by immunoblot analysis with antibodies against potential interacting proteins. However, in many cases, the only immunoreagents available for blotting are antibodies that have been generated in the same animal species as the antibodies used for immunoprecipitation. This can give rise to extremely high levels of background staining on blots because secondary antibody conjugates bind directly to the electrophoresed immunoglobulins in the immunoprecipitate. We have overcome this problem by probing immunoblots of immunoprecipitates with biotinylated antibodies, followed by an avidin-peroxidase conjugate.

We used rabbit antibodies against human ezrin (2) to demonstrate the difference between a conventional method and our improved method for detecting antigens in immunoprecipitates (Figure 1). Ezrin is an 81-kDa membrane-cytoskeletal linking protein (Figure 1, arrowhead) that is highly enriched in microvilli, which line the human placental trophoblast epithelium (1). Immunoprecipitates of ezrin were prepared from soluble extracts of isolated placental microvilli, separated by SDS-PAGE, transferred to membranes, and then stained using either unconjugated (Figure 1A) or biotinylated (Figure 1B) ezrin antibodies, followed by goat anti-rabbit IgG-peroxidase or avidin-peroxidase secondary reagents, respectively. In the conventional method, a major band at 81 kDa, corresponding to the mobility of ezrin, was easily observed in immunoprecipitates under reducing conditions (Figure 1A, lane 6). However, this band was not seen under non-reducing conditions (Figure 1A, lane 8) because the high levels of background staining on the blot occlude it. This background staining, which was particularly abundant in non-reduced samples, is due to the direct binding of the secondary goat anti-rabbit IgG to the rabbit immunoglobulins present in the Laemmli (3) sample of the immunoprecipitate (compare Figure 1A, lanes 5–8, and Figure 1A, lanes 5′–8′). In contrast,
the background staining was not seen when duplicate blots were probed with the avidin-peroxidase conjugate (Figure 1B, lanes 5′–8′) ExtrAvidin® (Sigma, St. Louis, MO, USA). Unlike the secondary antibody conjugate used in Figure 1A, the avidin-peroxidase conjugate used in Figure 1B did not recognize the electrophoresed immunoglobulins within the immunoprecipitate. Moreover, specific bands of ezrin were readily detected in immunoprecipitates using biotinylated ezrin antibody and the ExtrAvidin-peroxidase, regardless of whether samples were reduced or not (Figure 1B, lanes 6 and 8). Comparison of serial dilutions of total placental microvilli revealed that biotinylation of the affinity-purified ezrin antibody had little or no detectable effect on its sensitivity or specificity (compare Figure 1A, lanes 1–4, and Figure 1B, lanes 1–4). Moreover, we have found that the sensitivity and specificity of more than a half dozen other biotinylated rabbit polyclonal antibodies were retained on blots. Because a wide range of biotinylated antibodies from different species is available through commercial suppliers, we speculate that biotin-labeling will also be applicable to many monoclonal antibodies. However, the effect of biotinylation on antibody sensitivity or specificity must be determined empirically for each immunoreagent. Table 1 describes a procedure for biotinylation.

Although ezrin was detected in immunoprecipitates electrophoresed under reducing conditions using the conventional method for blotting, the considerable amount of background staining would preclude visualization of many other bands, especially in the region of the IgG-heavy chain (Figure 1A, asterisk). However, using the improved method for blotting, it is possible to detect polypeptides even when they co-migrate with the IgG-heavy chain (4). Thus, our improved method considerably expands the number of different-sized bands that can be visualized on immunoblots of immunoprecipitates. In addition, this method is simple, rapid, economical, and requires only small quantities of antibody.

REFERENCES


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