

Specific application

Comparison of indirect and direct detection procedures

Gill *et al.* (1996) have used the above immunoprecipitation procedure to demonstrate the difference in specificity between indirect and direct detection procedures for Western blot analysis. Briefly, they:

- 1 Expressed HA-tagged green fluorescent protein (HA-GFP) in *E. coli*
Note: The *A. victoria GFP* gene was provided by M. Chalfie, Columbia University (Chalfie *et al.*, 1994)
- 2 Immunoprecipitated HA-GFP using two amounts of *E. coli* lysates (5 μ l or 15 μ l of lysate containing 3.3 mg/ml total protein, diluted to 1 ml in lysis buffer) with 5 μ g of anti-HA antibody
- 3 Separated the proteins in the immunoprecipitates on an SDS-acrylamide gel and transferred them to a membrane by Western transfer
- 4 Detected the HA-GFP by two methods:
 - ▶ Indirect, using Anti-HA monoclonal antibody (1.0 μ g/ml) as primary antibody and goat Anti-Mouse IgG (H&L)-peroxidase conjugate as secondary antibody
 - ▶ Direct, using Anti-HA-peroxidase conjugate (0.1 μ g/ml)
- 5 Visualized the antibody-antigen conjugates with a chemiluminescent peroxidase substrate

The results, shown in Figure 4D.2, indicate the difference in specificity of the direct method over the indirect method. The bands visible in the indirect method include the heavy and light chains of Anti-HA antibody (recognized by the secondary antibody). Only the HA-GFP protein is visible in the direct detection procedure.

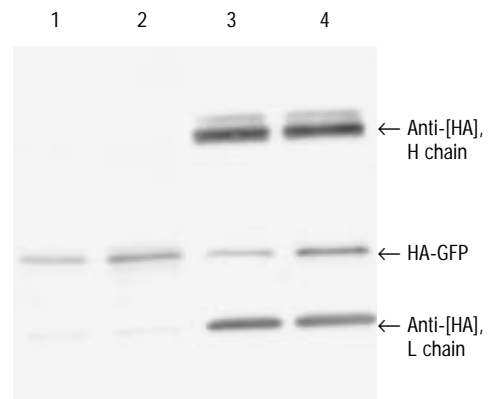


Figure 4D.2: Comparison of direct and indirect detection methods for HA-GFP on a Western blot. Details of the experiment are given in the text. Lanes 1 and 2: Immunoprecipitate from 5 μ l (lane 1) and 15 μ l (lane 2) of *E. coli* lysate, detected with Anti-HA-peroxidase (direct method). Lanes 3 and 4: immunoprecipitate from the same two lysates, detected with Anti-HA and Anti-Mouse IgG-peroxidase (indirect method).

References

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