0273-2289/93/3803—0243$03.60

Erasure of Western Blots after Autoradiography or Chemiluminescent Detection

SCOTT H. KAUFMANN* AND JOEL H. SHAPER

Oncology Center, Johns Hopkins Hospital and Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, MD

INTRODUCTION

Western blotting (reviewed in *1–3*) refers to formation and detection of an antibody-antigen complex between an antibody and a polypeptide that is immobilized on derivatized paper. Most commonly, polypeptides in a complex mixture are separated by electrophoresis through polyacrylamide gels in the presence of sodium dodecylsulfate (SDS), electrophoretically transferred to thin sheets of nitrocellulose or nylon, and reacted sequentially with one or more antibody-containing solutions. This sequence of manipulations can be utilized to determine whether a polypeptide recognized by a specific antiserum is present in a particular biological sample (cell type, subcellular fraction, or biological fluid), to follow the purification of the polypeptide, or to assess the location of epitopes within the polypeptide during chemical or enzymatic degradation. Alternatively, the same series of manipulations can be utilized to determine whether antibodies that recognize a particular polypeptide are detectable in a sample of biological fluid. Since Western blotting takes advantage of the power of electrophoresis for separating complex mixtures of polypeptides, it is possible to derive large amounts of information from this technique without necessarily purifying the antigen being studied.

This article is based on a chapter that first appeared in *Methods in Molecular Biology, Vol. 10: Immunochemical Protocols* Ed.: M. Manson ©1992 The Humana Press, Inc., Totowa, NJ.

*Author to whom all correspondence and reprint requests should be addressed.

There are certain circumstances in which it is convenient to be able to dissociate the antibodies from a Western blot after detection of antibody–antigen complexes. If, for example, the experiment gives an unexpected result regarding the subcellular distribution of a polypeptide, it is convenient to be able to reprobe the blot with an antibody that recognizes a second polypeptide in order to confirm that the samples have been properly prepared, loaded, and tranferred. Likewise, if the polypeptides being analyzed are derived from a precious source (e.g., biological fluid, tissue, or organism that is not readily available), it is convenient to dissociate the antibody–antigen complexes and reutilize the blots.

Over the last decade, several methods for removing antibodies from Western blots have been described. In early experiments, proteins were covalently bound to diazotized paper. Antibodies that were subsequently (noncovalently) bound to the paper were removed by treating the paper at 60°C with 10*M* urea (*4*) or 2% (w/v) sodium dodecylsulfate (*5*) under reducing conditions. Because of several undesirable properties (reviewed in *1–3*), diazotized paper has been largely replaced by nitrocellulose, a solid support to which proteins are noncovalently bound. It has been reported that treatment of nitrocellulose blots with glycine at pH 2.2 (*6*) or with 8*M* urea at 60°C (*7*) will remove antibodies and permit reuse of blots. Whereas these techniques are effective at disrupting low-affinity interactions between antigens and antibodies, they have been found to be ineffective at disrupting interactions between immobilized antigens and high-affinity antibodies (*6,8*).

Two subsequent observations have allowed the development of a more widely applicable technique for the removal of antibodies from Western blots. First of all, it was observed that treatment of nitrocellulose with acidic solutions of methanol would "fix" tranferred polypeptides to the nitrocellulose (reviewed in *3, see also 9,10*). Polypeptides treated in this fashion remained bound to the nitrocellulose even during treatment with SDS at 70–100°C under reducing conditions (*8,9*). Second, it was observed that removal of antibodies from nitrocellulose after Western blotting could be facilitated by reincubation of the blot with a large excess of irrelevant protein immediately prior to drying and autoradiography (*8*). Based on these observations, we present a technique that allows the reutilization of Western blots after reaction with a wide variety of antibodies or with lectins. In brief, polypeptides immobilized on nitrocellulose are stained with dye dissolved in an acidic solution of methanol. After unoccupied binding sites have been saturated with protein, the nitrocellulose is treated sequentially with unlabeled primary antibodies and radiolabeled secondary antibodies. Prior to drying, the blot is briefly incubated in a protein-containing buffer. After subsequent drying and autoradiography, the antibodies are removed by treating the nitrocellulose with SDS at 70°C under reducing conditions. A similar erasure procedure removes peroxidase-coupled antibodies after detection of antigens by enhanced chemiluminescence.

MATERIALS

1. Apparatus for transferring polypeptides from gel to solid support. (Design principles are reviewed in refs. *2,3.*)
   a. TE52 reservoir-type electrophoretic transfer apparatus (Hoefer Scientific, San Francisco, CA) equivalent.
   b. Polyblot semidry blotter (ABN, Hayward, CA) or equivalent.
2. Paper support for binding tranferred polypeptides.
   a. Nitrocellulose.
   b. Nylon (e.g., Genescreen from New England Nuclear, Boston, MA, or Nytran from Schleicher and Schuell, Keene, NH).
   c. Polyvinylidene difluoride PVDF (e.g., Immobilon; Millipore, Bedford, MA).
3. Fast green FCF, for staining polypeptides after transfer to solid support.
4. Penicillin 10,000 U/mL and streptomycin 10 mg/mL.
5. Reagents for electrophoresis (acrylamide, *bis*-acrylamide, 2-mercaptoethanol, sodium dodecylsulfate) should be electrophoresis grade.
6. All other reagents (Tris, glycine, urea, methanol) are reagent grade.
7. Transfer buffer: 0.02% (w/v) Sodium dodecylsulfate (SDS), 20% (v/v) methanol, 192 m*M* glycine-HCl, and 25 m*M* Tris base. Prepare enough buffer to fill the chamber of the transfer apparatus and a container for assembling the cassette.
8. TS buffer: 150 m*M* NaCl, 10 m*M* Tris-HCl, pH 7.4. This can be conveniently prepared as a 10X stock (1.5*M* NaCl, 100 m*M* Tris-HCl, pH 7.4). The 10X stock can be stored indefinitely at 4°C and then used to prepare 1X TS buffer, TSM buffer, and the other buffers described below.
9. TSM buffer: TS buffer containing 5% (w/v) powdered milk, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1 m*M* sodium azide. This buffer can be stored for several days at 4°C. Note that sodium azide is poisonous and can form explosive copper salts in drain pipes if not handled properly.
10. TS buffer containing 2*M* urea and 0.05% (w/v) Nonidet P-40. Prepare 300 mL per blot by combining 0.15 g of Nonidet P-40, 30 mL of 10X TS buffer, 75 mL of 8*M* urea (freshly deionized over Bio-Rad AG1X-8 mixed bed resin to remove traces of cyanate), and 195 mL of water.
11. TS buffer containing 0.05% (w/v) Nonidet P-40. Prepare 300 mL per blot.
12. Fast green stain: 0.1% (w/v) Fast green FCF in 20% (v/v) methanol-5% (v/v) acetic acid. This stain is reusable. Prepare 50–100 mL per blot.

13. Fast green destain: 20% (v/v) Methanol in 5% (v/v) acetic acid.
14. Blot erasure buffer: 2% (w/v) Sodium dodecylsulfate (SDS), 62.5 m*M* Tris-HCl (pH 6.8), and 100 m*M* 2-mercaptoethanol. The SDS/Tris-HCl solution is stable indefinitely at 4°C. Immediately prior to use, 2-mercaptoethanol is added to a final concentration of 6 μL/mL.
15. Primary antibody.
16. [^{125}I]-labeled secondary antibody. Secondary antibodies can be labeled as previously described (*8*) or purchased commercially. Radiolabeled antibodies should only be used by personnel trained to properly handle radioisotopes.
17. In lieu of radiolabeled secondary antibody, reagents for detection by chemiluminescence. This approach requires enzyme-coupled secondary antibody and a substrate that becomes chemiluminescent as a consequence of enzymatic modification, e.g., peroxidase-coupled secondary antibody and luminol (Amersham (Arlington Heights, IL) ECL enhanced chemiluminescence kit or equivalent).

METHODS

Transfer of Polypeptides to Nitrocellulose

The following description is appropriate for transfer in a transfer reservoir. If a semidry transfer apparatus is to be used, follow the manufacturers' instructions (*see* Note 1).

1. Perform SDS-polyacrylamide gel electrophoresis using standard techniques (*see* refs. *11,12* for a description of this method).
2. Wear disposable gloves while handling the gel and nitrocellulose at all steps. This avoids cytokeratin-containing fingerprints.
3. Cut nitrocellulose sheets to a size slightly larger than the polyacrylamide gel (*see* Note 2).
4. Fill the transfer apparatus with transfer buffer (*see* Note 3).
5. Fill a container large enough to accomodate the transfer cassettes with transfer buffer. Assemble the cassette under the surface of the buffer in the following order:
   a. Back of the cassette.
   b. Two layers of filter paper.
   c. The gel.
   d. One piece of nitrocellulose—gently work bubbles out from between the nitrocellulose and gel by rubbing a gloved finger or glass stirring rod over the surface of the nitrocellulose.

e. Two layers of filter paper—again, gently remove bubbles.
f. Sponge or flexible absorbant pad.
g. Front of the cassette.

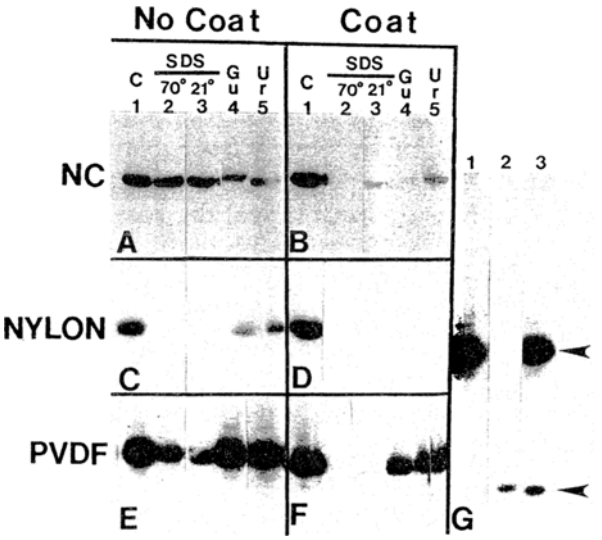
6. Place the cassette in the transfer apparatus so that the front is oriented toward the POSITIVE pole.
7. Transfer at 4°C in a cold room with the transfer apparatus partially immersed in an ice-water bath. Power settings: 90 V for 5–6 h or 60 V overnight.
8. Place the fast green stain in a container with a surface area slightly larger than one piece of nitrocellulose (*see* Note 4). After the transfer is complete, place all the pieces of nitrocellulose in the stain and incubate for 2–3 min with gentle agitation. Decant the stain solution, which can be reused (*see* Note 5).
9. Destain the nitrocellulose by rinsing it for 3–5 min in fast green destain solution with gentle agitation. Decant the destain (which can also be reused). Rinse the nitrocellulose four times (5 min each) with TS buffer (200 mL/rinse).
10. Mark the locations of lanes, standards, and any other identifying features by writing on the blot with a standard ball point pen.
11. Coat the remaining protein-binding sites on the nitrocellulose by incubating the blot in TSM buffer (50–100 mL per blot) for 6–12 h at room temperature (*see* Note 6). Remove the blot from the TSM buffer. Wash the blot four times in quick succession with TS buffer (25–50 mL per wash) and dry the blot on fresh paper towels. Either before or after coating of the unoccupied protein binding sites, blots can be dried and stored indefinitely in an appropriate container, e.g., Ziplock™ disposable food storage bags (1–2 blots/bag).

Detection of Antibody–Antigen Complexes Using Radiolabeled Secondary Antibody

12. Place the nitrocellulose blot in an appropriate container for reaction with the antibody. A 15- or 20-lane sheet can be reacted with 15–20 mL of antibody solution in a Ziplock™ bag. A 1- or 2-lane strip can be reacted with 2–5 mL of antibody solution in a disposable 15 mL conical test tube.
13. If the nitrocellulose has been dried, rehydrate it by incubation for a few minutes in an appropriate volume of TSM buffer (*see* previous step).
14. Add an appropriate dilution of antibody to the TSM buffer and incubate overnight (10–15 h) at room temperature with gentle agitation (*see* Notes 7 and 8).
15. Remove the antibody solution and save for reuse (*see* Note 9).

16. Wash the nitrocellulose with the following solutions (100 mL/wash for each large blot or 15–50 mL/wash for each individual strip): TS buffer containing 2*M* urea and 0.05% NP-40: three washes (15 min each); TS buffer: one wash (5 min) (*see* Note 10).
17. Add fresh TSM buffer to the nitrocellulose sheets or strips. For nitrocellulose sheets (or pooled strips) in Ziplock™ bags, it is convenient to use 50 mL of TSM buffer. Add 5–10 μCi [^{125}I]-labeled secondary antibody (*see* Note 11). Incubate for 90 min at room temperature with gentle agitation.
18. Remove the radiolabeled antibody and discard appropriately.
19. Wash the sheets as follows using 100 mL/wash for each large blot or for each group of pooled strips: TS buffer containing 0.05% NP-40: three washes (15 min each). Additional washes can be performed until no further radioactivity elutes in the wash as measured by a hand-held gamma counter. TS buffer: one wash (5 min).
20. Before drying blots, incubate them for 5 min with TSM buffer. This incubation step facilitates subsequent removal of antibody and reuse of the blot (*see* Note 12 and Fig. 1).
21. After incubating the blot with TSM buffer, immediately dry it between several layers of paper towels. After 5 min, move the blot to fresh paper towels to prevent the nitrocellulose from sticking to the paper towels. Allow the blot to dry thoroughly.
22. Mount the dried blot on heavy paper, cover it with clear plastic wrap, and subject it to autoradiography (*see* refs. *13,14* for details).

Fig. 1. *(opposite page)* Conditions for dissociating radiolabeled antibodies from Western blots after immobilization of polypeptides on various solid supports. Replicate samples containing 2×10^6 rat liver nuclei were subjected to polyacrylamide gel electrophoresis in the presence of SDS as previously described *(18)*. The separated polypeptides were transferred to nitrocellulose paper (panels A, B), Nytran nylon sheets (panels C, D), or Immobilon PVDF paper (panels E–G) (*see* Note 2). Unoccupied binding sites were blocked by incubation with milk-containing buffer (Step 11). Blots were incubated with chicken polyclonal antiserum that reacts with nuclear envelope polypeptide lamin B *(18)* followed by [^{125}I]-labeled rabbit antichicken IgG (Steps 12–19). One-half of each blot (panels B, D, F, and G) was coated with milk-containing buffer for 5 min prior to drying (Step 20); the other half of each blot was dried without being recoated with protein (panels A, C, and E). Audioradiography (not shown) confirmed that the signal in all lanes of a given panel was identical prior to subsequent manipulation. Panels A–F: To investigate the efficacy of various conditions for dissociating antibodies, samples were incubated at 70°C for 30 min with SDS erasure buffer (lane 2), with 6*M* guanidine hydrochloride in 50 m*M* Tris-HCl (pH 6.8) containing 100 m*M* 2-mercaptoethanol (lane 4), or with 8*M* urea in 50 m*M* Tris-HCl (pH 6.8) containing 100 m*M* 2-mercaptoethanol (lane 5). Alternatively, samples were incubated at



21°C for 30 min with SDS erasure buffer (lane 3). Strips were then washed twice with TS buffer and dried for autoradiography. Untreated strips (lane 1) served as controls. In each pair of panels, nonadjacent wells from a single autoradiograph have been juxtaposed to compose the figure. It is important to note that coating with milk prior to drying (Step 20) does not affect the amount of radiolabeled antibody initially bound to the blots (cf lane 1 in panels A and B, C and D, E and F). The efficacy of various treatments at removing antibodies varies depending on the solid support. For nitrocellulose or PVDF, coating of the blots with protein prior to drying (panels B, F) greatly facilitates the dissociation of antibodies. In both cases, SDS-containing buffer (lanes 2, 3) is more effective than guanidine hydrochloride (lane 4) or urea (lane 5) at dissociating the antibodies. For Nylon, SDS-containing buffer is again more effective at dissociating the antibodies (cf lanes 2–5 in panel C). When SDS-containing erasure buffer is used, it is not necessary to recoat nylon with protein prior to drying for autoradiography (cf lanes 2 or 3 in panels C and D). On the other hand, when guanidine hydrochloride-containing buffer is used to dissociate antibodies, it is necessary to recoat the nylon (cf lane 4 in panels C and D). Panel G: Reutilization of blots after dissociation of antibodies. Nuclear polypeptides were immobilized on PVDF, reacted with antibodies, and treated with milk-containing buffer (Step 20) prior to drying. Autoradiography (not shown) confirmed that all three lanes initially had indistinguishable signals for the 66 kDa lamin B polypeptide. After lane 2 was treated with SDS erasure buffer and recoated with milk (Steps 24–27), lanes 2 and 3 (a lane that was not erased) were reacted sequentially with chicken antiserum that recognizes the 38 kDa nucleolar polypeptide B23 (*18*) and [^{125}I]-labeled rabbit antichicken IgG. The signal for B23 (lower arrow) was readily detectable on the strip that had previously been erased (lane 2), as well as the strip that had not previously been erased (lane 3). Thus, treatment with SDS erasure buffer did not remove the nuclear polypeptides from the PVDF paper nor substantially alter their reactivity with polyclonal antibodies. The absence of a signal for lamin B after erasure (upper arrow, lane 2) indicates that the erasure buffer efficiently dissociated the antilamin B primary antibodies as well as the radiolabeled secondary antibodies from the PVDF-immobilized polypeptides.

Dissociation of Antibodies from Western Blots

23. After the blot has been subjected to autoradiography for the desired length of time, remove it from its mounting and place it in a Ziplock™ bag.
24. Add 50 mL of erasure buffer, seal the bag, and incubate in a water bath at 70°C for 30 min with gentle agitation every 5–10 min (*see* Notes 13 and 14).
25. Decant and discard the erasure buffer. Wash the blot twice (5 min each) with 50-100 mL of TS buffer to remove SDS.
26. To ensure that nonspecific binding sites on the blot are well coated, incubate with TSM buffer for 6–8 h at room temperature with gentle agitation.
27. The blot is ready to be stored (Step 11) or to be incubated with a new antibody as described in the previous section.

NOTES

Transfer of Polypeptides to Nitrocellulose

1. The method described is for transferring polypeptides after electrophoresis in SDS-containing polyacrylamide gels. Alternative methods have been described for transferring polypeptides after acid–urea gels and after isoelectric focusing (reviewed in 2,3).
2. Choice of solid support for polypeptides. Fig. 1 shows the results obtained when various solid supports (nitrocellulose, nylon, PVDF) are used for Western blotting, stripped of antibodies, and reused. Nitrocellulose (Figs. 1A,B) has the advantage of ease of use. It is compatible with a wide variety of staining procedures. With multiple cycles of blotting and erasing, however, nitrocellulose tends to become brittle. Derivatized nylon (Figs. 1C,D) has the advantage of greater protein binding capacity and greater durability, but avidly binds many nonspecific protein stains (reviewed in *1–3*). The higher binding capacity of nylon is said to contribute to higher background binding despite the use of blocking solutions containing large amounts of protein (reviewed in ref. *3*). Antibodies can be more easily dissociated from nylon than from nitrocellulose (cf Figs. 1C,A). PVDF membranes are durable, are compatible with a variety of nonspecific protein stains, and are capable of being stripped of antibody (Fig. 1F) and reutilized (Fig. 1G).

3. Various compositions of transfer buffer have been described (reviewed in *1–3*). Methanol is said to facilitate the binding of polypeptides to nitrocellulose, but to retard the electrophoretic migration of polypeptides out of the gel. In the absence of SDS, polypeptides with molecular weights above 116 kDa do not transfer efficiently. Low concentrations of SDS (0.01–0.1%) facilitate the transfer of larger polypeptides, but simultaneously increase the current generated during electrophoretic transfer, necessitating the use of vigorous cooling to prevent damage to the transfer apparatus.
4. Alternative staining procedures (reviewed in *1–3*) utilize Coomassie blue, Ponceau S, Amido black, India drawing ink, colloidal gold, or silver.
5. A washing step in acidified alcohol is probably essential to immobilize the polypeptides on the nitrocellulose (*3,9,10*). The fast green staining procedure satisfies this requirement. Polypeptides are observed to elute from nitrocellulose under mild conditions if a wash in acidified alcohol is omitted (*10,15*).
6. Various proteins have been utilized to block unoccupied binding sites on nitrocellulose (reviewed in *2,3*). These include 5% (w/v) powdered dry milk, 3% bovine serum albumin, 1% hemoglobin, and 0.1% gelatin. Blots of polypeptides immobilized on nitrocellulose have been successfully stripped of antibody and reutilized after coating of unoccupied binding sites with any of these protein solutions (*8*), if the blot is recoated with the protein solution immediately prior to drying (Step 20).

Formation of Antigen–Antibody Complexes

7. No guidelines can be provided regarding the appropriate dilution of antibody to use. Some antisera are useful for blotting at a dilution of greater than 1:20,000. Other antisera are useful at a dilution of 1:5 or 1:10. When attempting to blot with an antiserum for the first time, it is reasonable to try one or more arbitrary concentrations in the range of 1:10-1:500. If a strong signal is obtained at 1:500, further dilutions can be performed in subsequent experiments.
8. Different investigators incubate blots with primary antibodies for different amounts of time (reviewed in *3*). Preliminary studies with some of our antibodies have revealed that the signal intensity on Western blots is much greater when blots are incubated with antibody overnight rather than 1–2 h at room temperature (unpublished observations).

9. Diluted antibody solutions can be reused multiple times. They should be stored at 4°C after additional aliquots of penicillin/streptomycin and sodium azide have been added. Some workers believe that the amount of nonspecific (background) staining on Western blots diminishes as antibody solutions are reutilized. Antibody solutions are discarded when the intensity of the specific signal begins to diminish.
10. Choice of wash buffer after incubation with primary antibody: 2*M* urea is included in the suggested wash buffer to diminish nonspecific binding. Alternatively, some investigators include a mixture of SDS and nonionic detergent (e.g., 0.1% (w/v) SDS and 1% (w/v) Triton X-100) in the wash buffers. For antibodies with low avidity (especially monoclonal antibodies), the inclusion of 2*M* urea or SDS might diminish the signal intensity. These agents are, therefore, optional depending on the properties of the primary antibody used for blotting.
11. [^{125}I]-labeled protein A can be substituted for radiolabeled secondary antibody. Protein A, however, can bind to the immunoglobins present in milk (causing a high background on the blot). Hence, when [^{125}I]-labeled protein A is to be used, milk should not be utilized to block unoccupied binding sites (Step 11), nor as a diluent for antibodies (Steps 13, 14, and 17). Instead, bovine serum albumin, hemoglobin, or gelatin should be considered (*see* Note 6).

Dissociation of Antibodies After Autoradiography

12. Reincubation of blots with protein-containing buffer prior to drying has been found to be essential for efficient dissociation of antibodies from nitrocellulose (cf Figs. 1B and A) or PVDF paper (cf Figs. 1F and E). Recoating the blots is not required in order to dissociate antibodies from Western blots performed on certain types of nylon (Fig. 1C).
13. Choice of erasure buffer. Preliminary experiments have shown that the SDS/2-mercaptoethanol erasure buffer is more effective than urea, guanidine hydrochloride, or acidic glycine at dissociating polyclonal antibodies from Western blots on nitrocellulose (*8; see also* Fig. 1B) or PVDF (Fig. 1F). On the other hand, 6*M* guanidine hydrochloride is effective under certain conditions at removing antibodies from nylon (ref. *8* and Fig. 1D).
14. a. Temperature of incubation. When blotting is performed after immobilization of polypeptides on nitrocellulose, complete removal of antibodies requires heating of erasure buffer to $\geq$ 50°C for 30 min (*8; see also* Fig. 1B, lanes 2, 3).

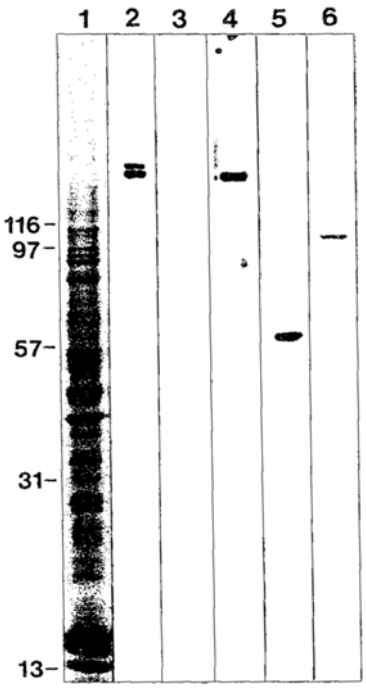


Fig. 2. Removal of antibodies after antigen detection by enhanced chemiluminescence. Replicate gel lanes containing protein from 3×10^5 HL-60 human leukemia cells were stained with Coomassie blue (lane 1) or tranferred to nitrocellulose. After unoccupied binding sites on the nitrocellulose were blocked by incubation with milk-containing buffer (Step 11), multiple identical strips (lanes 2–6) were reacted with antiserum directed against both isoforms of the nuclear enzyme topoisomerase II (ref. *19*), and washed with TS buffer containing 2*M* urea and 0.05% (w/v) NP-40 (Steps 12–16). The strips were then incubated for 60 min with peroxidase-coupled goat antirabbit IgG, washed with phosphate-buffered saline containing 0.05% (w/v) Tween 20, incubated with luminol, and covered with plastic wrap as described in ref. *17*. After detection of the chemiluminescence (lane 2), nitrocellulose strips were stored overnight. The blots were treated as described in Steps 23–27 to dissociate the antibodies and recoat any unoccupied binding sites. The strips were then incubated in TSM buffer without primary antibody (lane 3) or with rabbit antisera raised against the 170-kDa isoform of topoisomerase II (lane 4), the c-myc protein (lane 5, kindly provided by Chi Dang, Johns Hopkins University School of Medicine), or human topoisomerase I (lane 6, kindly provided by Leroy Liu, Johns Hopkins University School of Medicine). Washing and chemiluminescent detection were performed as described above. The absence of a signal at 180 kDa in lanes 3–6 confirms that the primary antibody used in the first detection step (lane 2) has been successfully removed.

In the case of blots subjected to chemiluminescent detection, the antibody removal technique is successful even though the strips were not recoated with protein-containing solution immediately prior to the detection step (Step 20). The need for recoating the blots (Step 20 and Note 12) is apparently obviated by covering the strips with plastic wrap and preventing drying during the detection and storage steps.

On the other hand, after immobilization of polypeptides on nylon, antibodies are efficiently dissociated by erasure buffer at room temperature (lane 3 in Figs. 1C,D).

b. Length of incubation. When blotting is performed on nitrocellulose, complete dissociation of antibodies at 70°C requires a minimum of 20 min incubation with erasure buffer (*8*). Incubation times for removal of antibodies from nylon and PVDF have not been investigated.

Removal of Antibodies After Chemiluminescent Detection

15. The technique described above is not useful for removing colored peroxidase reaction products (e.g., diaminobenzidine oxidation products) from blots. Thus, we avoid detection methods based on these reactions. On the other hand, peroxidase-based luminescent assays (*16*) do not deposit a chemical reaction product on the blot and are compatible with this erasure method (*17*). An example of the use of this erasure method (Steps 23–27) in conjunction with chemiluminescent detection is shown in Fig. 2.

General Notes

16. The techniques described above can be applied to the detection of glycoproteins by radiolabeled lectins. For this application, blots would be coated with albumin or gelatin, reacted with radiolabeled lectin (Steps 17–19), and recoated with albumin or gelatin (Steps 20, 21) prior to drying. After autoradiography, the radiolabeled lectin would be solubilized in warm SDS under reducing conditions (Steps 23–27).

REFERENCES

1. Gershoni, J. M. and Palade, G. E. (1983), *Anal. Biochem.* **131,** 1–15.
2. Beisiegel, U. (1986), *Electrophoresis* **7,** 1–18.
3. Stott, D. I. (1989), *J. Immunol. Methods* **119,** 153–187.
4. Renart, J., Reiser, J., and Stark, G. R. (1979), *Proc. Natl. Acad. Sci. USA* **76,** 3116–3120.
5. Gullick, W. J. and Lindstrom, J. M. (1982), *Biochemistry* **21,** 4563–4569.
6. Legocki, R. P. and Verma, D. P. S. (1981), *Anal. Biochem.* **111,** 385–392.
7. Erickson, P. F., Minier, L. N., and Lasher, R. S. (1982), *J. Immunol. Methods* **51,** 241–249.
8. Kaufmann, S. H., Ewing, C. M., and Shaper, J. H. (1987), *Anal. Biochem.* **161,** 81–95.

9. Parekh, B. S., Mehta, H. B., West, M. D., and Montelaro, R. C. (1985), *Anal. Biochem.* **148,** 87–92.
10. Salinovich, O. and Montelaro, R. C. (1986), *Anal. Biochem.* **156,** 341–347.
11. Laemmli, U. K. (1970), *Nature* **227,** 680–685.
12. Cooper, T. G. (1977), *The Tools of Biochemistry.* Wiley, New York, pp. 206–212.
13. Laskey, R. A. and Mills, A. D. (1977), *FEBS Lett.* **82,** 314–316.
14. Swanstrom, R. and Shank, P. R. (1978), *Anal. Biochem.* **86,** 184–192.
15. Lin, W. and Kasamatsu, H. (1983), *Anal. Biochem.* **128,** 302–311.
16. Leong, M. M. L., Fox, G. R., and Hayward, J. S. (1988), *Anal. Biochem.* **168,** 107–114.
17. ECL Western Blotting Protocols (1991), Amersham International plc, Amersham, UK.
18. Kaufmann, S. H. (1989), *J. Biol. Chem.* **264,** 19346–19355.
19. Kaufmann, S. H., McLaughlin, S. J., Kastan, M. B., Liu, L. F., Karp, J. E., and Burke, P. E. (1991), *Cancer Res.* **51,** 3534–3543.