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## Detection of Five Nanograms of Protein by Two-Minute Nile Red Staining of Unfixed SDS Gels

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We have shown that the fluorescent dye Nile red can be used for the staining of protein bands in sodium dodecyl sulfate (SDS)-polyacrylamide gels (3). Unlike the current methods using Coomassie blue or silver (5,7,9,10), Nile red staining does not require the fixation of protein in the gel. This allows the direct electroblotting and further sequencing and immunodetection of protein bands (1). Nile red was used previously for the fluorescent staining of hydrophobic structures such as lipid droplets (6). In the case of proteins separated in SDS gels, Nile red interacts presumably with the hydrophobic core of SDS-protein complexes (4,8).

In the current staining method developed in our laboratory (2,3), the bands are visualized, with a detection limit of 20 ng of protein per band, after 5-min staining with Nile red. In this work, we have carried out a detailed study in order to reduce the background fluorescence of Nile red stained gels. The modified method presented in this report has a higher sensitivity and requires a shorter staining time than the original procedure.

Protein electrophoresis was run on a Mini-Protean<sup>®</sup> II apparatus (Bio-Rad, Hercules, CA, USA). To prevent the formation of pure SDS micelles in the gel (3,4), the stacking and separating gels (8 × 6 × 0.075 cm) and the running buffer contained 0.05% SDS. Following electrophoresis, the gel was stained with different staining solutions (see below) containing different amounts of Nile red (Sigma Chemical, St. Louis, MO, USA). In all experiments, the required volume of a concentrated solution of Nile red (0.4 mg/mL) in dimethyl sulfoxide (DMSO) was added to 50 mL of deionized water or the indicated solutions previously placed in a polypropylene box (12 × 7.5 × 7 cm). The mixture was agitated gently for about 5 s, the gel was immersed immediately in the resulting solution, and the

plastic box wrapped with aluminum foil was agitated on an orbital shaker (at about 150 rpm) for the times indicated. To obtain a homogeneous staining, care was taken to ensure that the gel was completely covered with the staining solution. Unless otherwise indicated, the staining was performed at room temperature. After staining, the gel was briefly rinsed with water (for

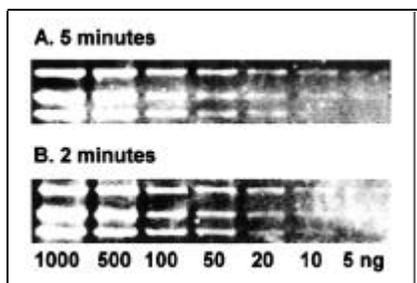
about 10 s) and finally visualized using an ultraviolet (UV) transilluminator (302 nm) and Polaroid film (Cambridge, MA, USA) or a charged-coupled device (CCD) integrating camera and the Molecular Analyst<sup>®</sup> software of the Gel Doc<sup>™</sup> 1000 system (Bio-Rad). Generally, images were obtained with integration times ranging from 4–6 s. Longer integration times (8–12

s) were used to obtain the maximum sensitivity; in this case image analysis was necessary in order to eliminate the background produced by the UV lamps of the transilluminator. The other experimental details are the same indicated in the original Nile red staining method described elsewhere (2,3).

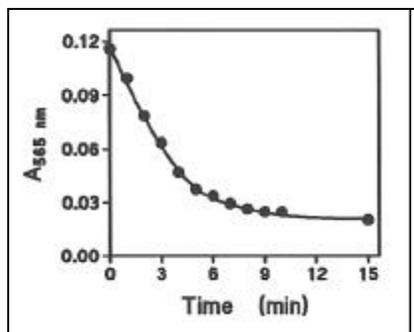
We have assayed staining protocols with different amounts of Nile red in water or in solutions containing different concentrations of methanol, DMSO, acetic acid, trichloroacetic acid and formic acid. The best results were obtained using water and lowering the Nile red concentration until no visible precipitate was observed after gel staining. With the staining conditions indicated in Figure 1A (5 min of staining with 2  $\mu\text{g}/\text{mL}$  of Nile red), the low background fluorescence allows the detection of as little as 5 ng of protein per band using the CCD camera with an 8-s integration time. By using Polaroid film, we were able to detect 10 ng of protein per band. These results were obtained at room temperature. Higher temperatures (60° and 100°C) during the staining do not significantly improve the detection limit of the method. With the same concentration of Nile red, but in the presence of 7.5% (vol/vol) acetic acid, we also obtained a high sensitivity. By using long integration times in the CCD camera, we can detect 5 ng of protein per band. However, this result is obtained after a relatively long staining time (at least 40 min). Moreover, in this case, the presence of acetic

acid produces protein precipitation, which is not convenient because it precludes protein transfer to membranes and further analyses (1). The other solutions analyzed in this study show less sensitive staining properties.

These results indicate that water is the most convenient solvent for Nile red staining. Although, with the very low concentration of Nile red used in this work, we are unable to see any precipitate at the end of the staining process, the analysis of the absorbance of Nile red in water, under the same conditions used for gel staining (Figure 2), shows a remarkable decrease in the amount of soluble Nile red in the first few minutes after the preparation of the staining solution. This observation explains why long staining times do not improve the sensitivity of the method (results not shown). Furthermore, the results presented in Figure 2 suggest that the staining time can be shortened. In fact, we have observed that 30 s of staining is enough to visualize 50 ng of protein per band. As shown in Figure 1B, a 2-min staining has approximately the same sensitivity found after staining for 5 min (Figure 1A). These conditions and the fact that the low background makes negligible the rinsing time necessary after staining demonstrate that the improved Nile red staining procedure described in this paper is very convenient for the fast and simple detection of protein bands in SDS gels with a high sensitivity.



**Figure 1.** Nile red staining of protein bands in 0.05% SDS-polyacrylamide gels. The gel was stained as described in the text with a solution prepared by the addition of 250  $\mu\text{L}$  of the stock solution of Nile red (0.4 mg/mL in DMSO) to 50 mL of deionized water. Staining was for (A) 5 and (B) 2 min. In both cases, the proteins were, from top to bottom, bovine serum albumin, ovalbumin and glyceraldehyde-3-phosphate dehydrogenase. The amount of protein per band is indicated at the bottom of each lane.



**Figure 2.** Time course of Nile red absorbance at 565 nm in the staining solution. A solution containing 2  $\mu\text{g}/\text{mL}$  of Nile red in water was prepared in a polypropylene box as described in the text for the staining of gels. The box without the gel was protected from light with aluminum foil and agitated continuously at 150 rpm. At different times, aliquots were withdrawn, and the absorbance of each aliquot was measured immediately at 565 nm.

## REFERENCES

1. Bermudez, A., J.-R. Daban, J.R. Garcia and E. Mendez. 1994. Direct blotting, sequencing and immunodetection of proteins after five-minute staining of SDS and SDS-treated IEF gels with Nile red. *BioTechniques* 16:621-624.
2. Daban, J.-R., S. Bartolome and A. Bermudez. 1996. Rapid Staining of proteins in polyacrylamide gels with Nile red, p. 179-185. In J.M. Walker (Ed.), *The Protein Protocols Handbook*. Humana Press, Totowa, NJ.
3. Daban, J.-R., S. Bartolome and M. Samsó. 1991. Use of the hydrophobic probe Nile red for the fluorescent staining of protein bands in sodium dodecyl sulfate-polyacrylamide gels. *Anal. Biochem.* 199:169-174.
4. Daban, J.-R., M. Samsó and S. Bartolome. 1991. Use of Nile red as a fluorescent probe for the study of the hydrophobic properties of protein-sodium dodecyl sulfate complexes in solution. *Anal. Biochem.* 199:162-168.
5. Garfin, D.E. 1990. One-dimensional gel electrophoresis, p. 425-441. In M.P. Deutscher (Ed.), *Methods in Enzymology*, Vol. 182. Academic Press, New York.
6. Greenspan, P., E.P. Mayer and S.D. Fowler. 1985. Nile red: A selective fluorescent stain for intracellular lipid droplets. *J. Cell. Biol.* 100:965-973.
7. Hames, B.D. 1990. One-dimensional polyacrylamide gel electrophoresis, p. 1-147. In B.D. Hames and D. Rickwood (Eds.), *Gel Electrophoresis of Proteins. A Practical Approach*. IRL Press, Oxford.
8. Samsó, M., J.-R. Daban, S. Hansen and G.R. Jones. 1995. Evidence for sodium dodecyl sulfate/protein complexes adopting a necklace structure. *Eur. J. Biochem.* 232:818-824.
9. Wirth, P.J. and A. Romano. 1995. Staining methods in gel electrophoresis, including the use of multiple detection methods. *J. Chromatogr. A* 698:123-143.
10. Zewert, T.E. and M.G. Harrington. 1993. Protein electrophoresis. *Curr. Opin. Biotechnol.* 4:3-8.

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