Enhanced Digital Imaging of Diaminobenzidene-Stained Immunoblots

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Diminution of minor immunoreactive bands to the extent that it may cause them to be excluded from quantitation is a problem frequently encountered when imaging Western blots. The sensitivity of such immunoanalyses is often attenuated by the incapacity of some digital scanners to detect threshold signals (2). Recently, Donovan et al. (1) described the use of amber-colored acetate transparencies as inexpensive optical filters for enhancing digital scans of purple-colored bands on Western blots produced by 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium (BCIP/NBT) color development of alkaline phosphatase-conjugated probes. Similarly, we use a green acetate transparency (MultiCraft, Knoxville, TN, USA) and using ScanWizard 3.0.6 and MicroScan 1.0.5 software (both from Microtek). IMAGE 1.55 software (NIH, Bethesda, MD, USA) supplemented with macros written by BioImaging Technologies (Brookfield, WI, USA) was used for image analysis.

As illustrated in Figure 1, sensitivity of the scanner was increased approximately threefold using the green acetate transparency as evidenced by the shifted abscissa of the inflection point of each plot. Green plastic wrap (dashed line) also increased scanner sensitivity, but only moderately and lacked the optical clarity necessary to be of much utility as an optical filter. Use of the acetate transparency increased sensitivity of the scanner to 0.3 ng/mm², near the detection limit of this particular immunoassay and clearly visible with the unaided eye, whereas only 0.6 ng/mm² could be recorded on the digital image without the use of an optical filter. Moreover, the shift in the nearly linear segments of the sigmoid plots of Figure 1 demonstrates that filter selection can extend the range for optimal assessment of concentrations. We suspect that attenuating the light absorption through use of a yellow or red filter would have enabled quantitation of high levels of antigen in the range of 10–40 ng/mm².

In conclusion, use of a green acetate transparency significantly increased sensitivity of the scanner for the detection of antigens on DAB-stained immunoblots. Green acetate transparencies thus offer a reasonable alternative to the considerably more expensive gelatin filters. Furthermore, the useful linear range for quantitation can potentially span two orders of magnitude when duplicate scans made with and without the green acetate transparency are compared.

REFERENCES


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