β -Naphthyl Acetate in Acetone Produces a Dark Background for Staining of Esterase Activity on Gel

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Abbreviations

BNA β -Naphthyl acetate
PAGE Polyacrylamide gel electrophoresis

Dear editor,

Staining of gels for esterase activity is a routinely performed procedure in any biological laboratory. The substrates popularly known for such purpose are α -naphthyl acetate or β -naphthyl acetate (BNA). After the electrophoresis run the gels are incubated with the ester substrates, and azo dye like Fast Red B. The separated protein having esterase function that cleaves the ester bond of the substrate concerned and forms either α -naphthol or β -naphthol which is subsequently stained by the azo dye. By the above procedure esterase activity can be easily visualised on gel [1].

As on date, there is no consensus about the preparation of the ester substrate. Earlier workers have dissolved the substrates in acetone or ethanol and detected the enzyme activity [2–5]. While working in this line, we have observed that if BNA is dissolved in acetone, it produces a dark background compared to the situation when the substrate is prepared by dissolving in ethanol. Enzyme activity bands are visible in both the cases [Fig. 1A, B for agarose and native polyacrylamide gel electrophoresis (PAGE) respectively; for methods, please refer the supplementary]. It is needless to emphasise that low background staining is a prerequisite for electrophoresis experiment.

It is interesting to note that destaining is sometimes necessary when the ester substrates are prepared in acetone [5]. This supports our observation that acetone as a dissolving medium of the ester substrate produces a dark background in the gel. With ethanol using BNA we have observed no (or very light) background staining. So if ethanol is used as a dissolving medium of BNA for esterase staining of gels, no destaining will be necessary.

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A

B

◀**Fig. 1 A** Representative picture of agarose gel electrophoresis of serum proteins followed by staining with BNA for detection of esterase activity. The gel is incubated with (a) 75 μ M BNA (in ethanol), (b) 75 μ M BNA (in acetone), (c) 1 mM BNA (in ethanol), (d) 1 mM BNA (in acetone). The densitometry analysis of the background of the gel ($\rightarrow$). Mean $\pm$ SD ($n = 6$) of the background density is shown in (e) for (a) & (b) and (f) for (c) & (d) respectively (Y-axis in square pixel where 96 pixel = 1 inch). *** p value less than 0.0001. **B** Representative picture of native PAGE of plasma proteins followed by staining with BNA for detection of esterase activity. The gel is incubated with (a) 75 μ M BNA (in ethanol), (b) 75 μ M BNA (in acetone), (c) 1 mM BNA (in ethanol), (d) 1 mM BNA (in acetone). The densitometry analysis of the background of the gel ($\rightarrow$). Mean $\pm$ SD ($n = 6$) of the background density is shown in (e) for (a) & (b) and (f) for (c) & (d) respectively (Y-axis in square pixel where 96 pixel = 1 inch). *** p value less than 0.0001

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Compliance with Ethical Standards

Conflict of interest Authors do not have any conflict of interest.

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