

Fisher, C. R. Anilinonaphthalene sulfonate and its magnesium and sodium salts as fluorescent protein stain.

Hartman and Udenfriend (1969 Anal. Biochem. 30:391) described a method for utilizing the mognerium salt of 1-anilino-8-naphthalene sulfonate (Eastman Organic Chemicals) as a rapid means for visualization of protein bands in acrylamide gels. The advantages of this technique are that the proteins within the gel are not denatured and that the bands can be cut out, eluted, and assayed for enzyme activity in a few minutes' time. An attempt to obtain this compound showed that it was no longer available commercially, but Eastman Organic agreed to supply a sample of the free acid and its sodium salt, and to prepare a sample of the mognerium salt for evaluation.

The technique used was basically that described by the previous authors. Aqueous solutions (1 mg/ml) of each of the compounds were prepared and dilutions were made in 0.1 M potassium phosphate buffer, pH 6.8, to give final concentrations of 0.003%. The gels were prepared in the ORTEC pulsed-power vertical gel electrophoresis apparatus, with a sample of 10-100 µg of protein per band. The gels were immersed for approximately 1 min. in the stain and then were viewed under a Mineral Light UV source. With this treatment, bands containing 50 µg of protein were easily visible when stained with the magnesium salt and were faintly visible with the sodium salt and the free acid.

By immersing the gel in 1 N HCl for a few seconds, rinsing in water, and restaining, we were able to detect 10-µg bands with the mognerium salt, 20-µg bands with the sodium salt, and 50-µg bands with the free acid. In all tests, the magnesium salt was markedly superior both to the sodium salt and to the free acid. The storage conditions for the reagents are quite important. Both the stock solutions and the diluted stains were greatly inactivated by exposure to light for a few days. Storage in amber bottles with refrigeration prevented any detectable deterioration over a period of several weeks.

By combining this technique with modifications in the electrophoresis gel (described in the following communication), we were able to go from virtually no recovery to a negligible loss of enzyme activity. The mognerium salt, although not listed in the catalog, is now available from Eastman Organic Chemicals as catalogue number 10990. This material has already been recrystallized and requires no further treatment before use. (This research was sponsored by the U. S. Atomic Energy Commission under contract with Union Carbide Corporation and by the AEC Postdoctoral Fellowship Program of the Oak Ridge Associated Universities. • • • Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.)