

# This Week's Citation Classic

CC/NUMBER 40  
OCTOBER 3, 1983

**Chrumbach A, Reisfeld R A, Wyckoff M & Zaccari J. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis.**

*Anal. Biochem.* 20:150-4, 1967.

[Endocrinology Branch, Natl. Cancer Inst., and Lab. Immunology, Natl. Inst. Allergy and Infectious Diseases, Natl. Insts. Health, Bethesda, MD]

A staining procedure for protein zones on polyacrylamide gel is described which a) provides a clear background without destaining; b) requires only 1.5 hours; c) maintains the protein band throughout in the reliable fixative, 12.5 percent trichloroacetic acid (TCA); and d) is sensitive to 2  $\mu$ g of protein per zone. [The SCI® indicates that this paper has been explicitly cited in over 1,035 publications since 1967.]

A. Chrumbach

Department of Health & Human Services  
National Institutes of Health  
Bethesda, MD 20205

July 8, 1983

"When I joined the National Institutes of Health in 1966, Ralph Reisfeld and I were the sole full-time devotees of polyacrylamide gel electrophoresis at that institution. June Zaccari and Mary Wyckoff were his and my assistants, respectively. Working with the glycoprotein, human chorionic gonadotropin (hCG), I found it impossible to fix and stain it with the standard procedure at that time, using one percent Amidoblack in 7.5 percent acetic acid as the stain-fixative. Ralph had a similar interest in improving fixation and staining of IgG chains, parathyroid hormone, pokeweed mitogen, and phytohemagglutinin. We decided to undertake a joint systematic search for a stain effective in all of those cases. A matrix of stain-fixative combinations was set up, including the stains Coomassie Brilliant Blue R-250, Amidoblack, Ponceau Red, Bromphenolblue, and Fluorescein, and the fixatives picric, perchloric, acetic, sulfosalicylic, tannic, phosphotungstic, and trichloroacetic acid (TCA) as well as mercuric chloride.

"TCA proved the only fixative capable of fixing hCG bands at least for a few hours and

was compatible with one of the dyes—Coomassie Blue R-250. Testing TCA at various concentrations, we made the chance observation that Coomassie Blue solutions in ten to 12.5 percent TCA stained without an appreciable background, while both lower and higher concentrations of TCA gave a background stain. This is presumably due to the fact that in this narrow range, the dye solution is saturated and partitions into the relatively hydrophobic protein zone. This view is supported by the fact that Coomassie Blue R-250 is partially insoluble in ten percent TCA, and precipitates after about one day from solution, temporarily brought about by rapid mixing of a solution of the dye in 20 or 25 percent TCA with an equal volume of water (a crucial recipe omitted through oversight from the report but provided later in footnote 4 of reference 1). The report is marred by as yet another accidental omission, i.e., failure to warn against the shrinkage of gels after two weeks of storage. Prolonged storage of the gels stained by the reported procedure is important because protein zones on gels stored in ten percent TCA containing a small amount of the dye take up progressively more Coomassie Blue R-250. Thus, storage can be used to increase detection sensitivity.

"If in spite of these oversights the report proved popular, this is undoubtedly due to the fact that it presented the first no-background, rapid staining procedure in polyacrylamide gel electrophoresis. Since that time, the procedure has been developed in two ways: 1) Substitution of Coomassie Blue R-250 by G-250 allows for preparing a stable 0.25 percent stock solution of the dye.<sup>2</sup> That procedure has the disadvantage, however, that storage is in the poor fixative, acetic acid, and that a band intensification with storage time is not achieved. 2) In conjunction with a rapid diffusion of SDS from gels, it allows for the staining of SDS-proteins within a few hours without a background."<sup>3</sup>

1. Rodbard D & Chrumbach A. Estimation of molecular radius, free mobility, and valence using polyacrylamide gel electrophoresis. *Anal. Biochem.* 40:95-134, 1971. (Cited 430 times.)
2. Diezel W, Kopperschlaeger G & Hofmann E. An improved procedure for protein staining in polyacrylamide gels with a new type of Coomassie Brilliant Blue. *Anal. Biochem.* 48:617-20, 1972. (Cited 235 times.)
3. An der Lan B C, Sullivan J V & Chrumbach A. Effective fixation and rapid staining of proteins in SDS-PAGE. (Sathakos D, ed.) *Electrophoresis '82*. Berlin: Walter de Gruyter, 1983. p. 225-33.