Detection of glutathione reductase after electrophoresis on either native or sodium dodecylsulfate polyacrylamide gels

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Abstract

Commercial glutathione reductase (GR) from spinach and yeast (Saccharomyces cerevisiae) were stained on 7.5% native polyacrylamide gel electrophoresis (PAGE) gels or 15% sodium dodecyl sulfate (SDS)-PAGE gels with or without further purification by a 2',5'-ADP Sepharose 4B affinity column. For SDS-PAGE gels, the SDS was removed first by washing twice with 25% isopropanol in 10 mM Tris-HCl (pH 7.9) for 10 min. The gel was then dipped in a 50 mM Tris-HCl buffer (pH 7.9) containing 4.0 mM oxidized glutathione (GSSG), 1.5 mM NADPH, and 2 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) for 20 min. The GR activity was negatively stained in the dark by a solution containing 1.2 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 1.6 mM phenazine methosulfate (PMS) for 5-10 min. The contrast between the clear zone of GR activity and the purple background was found in both native and SDS-PAGE gels. This negative staining method can detect GR as little as 0.064 units and 0.0032 units, respectively, for spinach and yeast sources. Under reduced SDS-PAGE gels, the GR activity band located on 72 kDa for spinach and 51 kDa for yeast. This fast and sensitive method could be used during enzyme purification and for characterization of GR from different sources under different physiological stages or conditions.