

Colorimetric assays help monitoring the protein function during the protein purification under denaturing conditions that use chaotic agents

Indirakumari Ruda^{1,2} Balarohitha Sundaram^{1,3} and Ravikiran S. Yedidi^{1,3,*}

¹Department of Intramural research core, The Center for Advanced-Applied Biological Sciences & Entrepreneurship (TCABS-E), Visakhapatnam 530003, A.P. India; ²Department of Biochemistry, Andhra University, Visakhapatnam 530003, A. P. India; ³Department of Biotechnology, Andhra University, Visakhapatnam 530003, A. P. India (*Correspondence to R.S.Y.: tcabse.india@gmail.com).

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Reporter genes play a pivotal role in molecular biology because they allow for real-time monitoring of protein expression and cellular response. Among these, Horseradish Peroxidase (HRP) is widely used because of its high sensitivity, stability, and ease of detection. Urea is a well-known protein denaturant that was hypothesized to impact HRP activity by disrupting its structure. This study aimed to evaluate the enzymatic activity of HRP under denaturing conditions, specifically focusing on the effects of urea at various concentrations. The results show that when urea concentrations increased, enzymatic activity dropped progressively, which is confirmed by the visual colour change of samples and also by analysing the samples by employing the digital technique ImageJ. These findings indicate that urea produces structural changes in the enzyme active site, compromising its catalytic effectiveness. Further, conformational changes can be monitored by using the various tools, like circular dichroism (CD), UV-vis spectroscopy, and fluorescence spectroscopy.

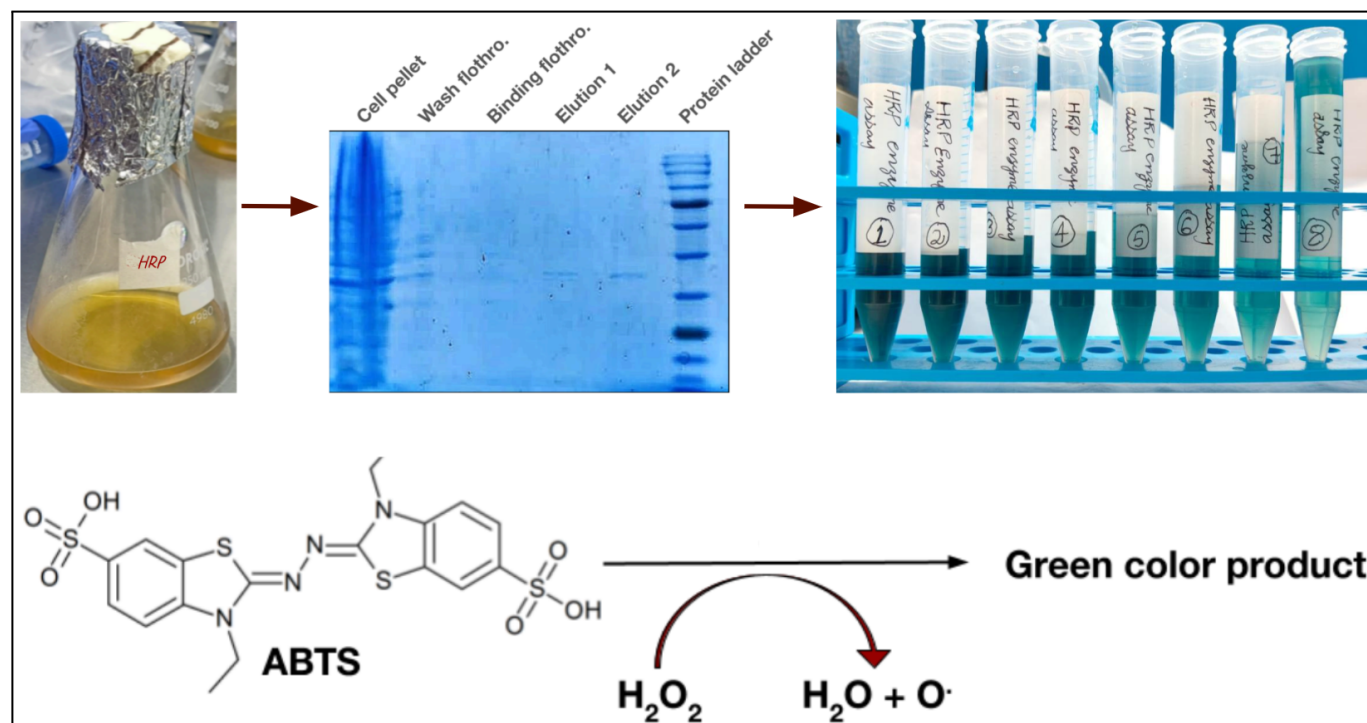


Figure 1. Overall process of expression, purification and denaturation studies of HRP.

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