QUICK PROTEIN QUANTIFICATION USING COOMASSIE INFRARED FLUORESCENCE AND ITS COMPATIBILITY WITH IMMUNODETECTION

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Coomassie Brilliant Blue stains have been studied for nearly four decades, and laboratories rely on them for visualizing proteins in acrylamide gels. Quantification generally is accomplished by densitometry of the visible blue color. Recently, Coomassie R-250 was found to have greatly enhanced fluorescence (720 nm max) when bound to protein and little or no fluorescence alone, and this complex can be detected in the 700 nm channel of the LI-COR Odyssey infrared fluorescence scanner. To capitalize on the ability to read and quantify fluorescence of Coomassie stained proteins, we have developed a quick staining procedure which takes only 30 minutes to complete. The method is sensitive: bands containing as little as 10 ng can be detected. The quantitative range is 10 ng to 20 ug. Since test proteins give similar integrated fluorescence intensity per unit mass, a single protein standard can be used for estimates of protein content in a band on an SDS-PAGE gel, and standards of a specific protein will allow precise protein measurements. Quick Coomassie staining is compatible with subsequent quantitative immunodetection using an IR800 dye linked secondary antibody, which can be detected in the 800 nm channel of Odyssey scanner, either in-gel, with a small loss of signal, or after blotting to PVDF or nitrocellulose. This new technique may contribute a quantitative and sensitive approach for investigation of the age-related protein profiles change or the specific protein modifications in cell cultures.