Detection of protease inhibitors by a reverse zymography method, performed in a tris(hydroxymethyl)aminomethane-Tricine buffer system

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Abstract: A new detecting method for protease inhibitors, especially for low-molecular-weight inhibitors, is reported. Inhibitor samples were separated on a protein substrate-SDS-polyacrylamide gel in a Tris-Tricine buffer system that improves the separation and identification of peptides and low-molecular-weight proteins. After electrophoresis, the gel was incubated with the target proteases to hydrolyze the background protein substrate. The inhibitor bands, which were protected from proteolysis by the target proteases, were stained. Standard low-molecular-weight inhibitors, such as pepstatin A for pepsin or matrix metalloproteases inhibitor I for collagenase, as well as larger inhibitors, such as soybean trypsin inhibitor or aprotinin for tryspin and cystatin C for papain, were demonstrated by this method and showed clear blue inhibitor bands in the white background when the gels were treated with the target proteases. Some significant applications of this method are introduced. This method is an ideal system for discovering new protease inhibitors in small natural samples.

Author Keywords: Protease inhibitors; Reverse zymography

Index Keywords: aprotinin; buffer; collagenase; cystatin C; matrix metalloproteinase inhibitor; methane; papain; pepsin A; pepstatin; peptide; proteinase; proteinase inhibitor; soybean trypsin inhibitor; tris(hydroxymethyl)aminomethane tricine; trypsin; unclassified drug; analytic method; article; controlled study; gel; human; hydrolysis; molecular weight; polyacrylamide gel electrophoresis; priority journal; protein analysis; protein degradation; protein determination; separation technique; staining; zymography; Buffers; Electrophoresis, Polyacrylamide Gel; Endopeptidases; Glycine; Protease Inhibitors; Proteins; Staining and Labeling; Tromethamine; Glycine max

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