

Reverse zymography using fluorogenic substrates for protease inhibitor detection

Le Q.T., Ohashi A., Hirose S., Katunuma N.

Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan; Biotechnology Center, Vietnam National University, Hanoi Xuan thuy-Cau giay, Hanoi, Viet Nam

Abstract: A novel, sensitive method for detecting protease inhibitors by using fluorescent protease substrates in gels is described. The protease inhibitors were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels containing a copolymerized peptide substrate, namely 4-methyl-coumaryl-7-amide (MCA). As the incorporated substrates in the gel, Boc-Phe Ser-Arg-MCA was used for trypsin, Suc-Ala-Ala-Pro-Phe-MCA for α -chymotrypsin, and Z-Phe-Arg-MCA for papain. After electrophoresis, washing and incubating the gel with the target protease solutions allowed the substrate to be cleaved by the protease, and the release of the fluorescent 7 amino-4 methyl-coumarin (AMC), which was detected under a UV transilluminator. The uncleaved peptide-MCA substrate remained where the inhibitors were present, and was visualized as dark blue bands on the light-green fluorescent background gel. This new method offers several advantages over other previous methods including: (i) greatly increased sensitivity can be achieved in a shorter period of time, which may be useful for discovering new protease inhibitors in small amounts of crude material; (ii) the procedure is quite simple and quick since the incubation period is very short and no time is needed for staining and destaining steps; (iii) since these probes using substrate specificity/target proteases, they are excellent tools for detection and discrimination of unknown protease inhibitors for various target proteases. © 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Author Keywords: Fluorogenic substrate; Protease inhibitors; Reverse zymography

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Manufacturers: Sigma, United States

Correspondence Address: Katunuma, N.; Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan; email: katunuma@tokushima.bunri-u.ac.jp

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Authors with affiliations:

- Le, Q.T., Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan, Biotechnology Center, Vietnam National University, Hanoi Xuan thuy-Cau giay, Hanoi, Viet Nam
- Ohashi, A., Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan
- Hirose, S., Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan
- Katunuma, N., Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan

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