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 ?-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

Index Keywords: 4 methylcoumaryl 7 amide; amino acid derivative; aprotinin; benzyloxycarbonylphenylalanylarginine 4 methylcoumaryl 7 amide; chymotrypsin A; chymotrypsin inhibitor; coumarin derivative; cystatin C; fluorescent dye; papain; polypeptide; proteinase; proteinase inhibitor; serine proteinase inhibitor; soybean trypsin inhibitor; succinylalanylalanylprolylphenylalanine 4 methylcoumaryl 7 amide; tert butyloxycarbonylphenylalanylserylarginine 4 methylcoumaryl 7 amide; trypsin; unclassified drug; article; drug determination; drug discrimination; electrophoresis; enzymatic assay; enzyme specificity; enzyme substrate; fluorescence; protein degradation; reverse zymography; zymography; Animals; Chymotrypsin; Coumarins; Electrophoresis, Polyacrylamide Gel; Fluorescent Dyes; Intestinal Mucosa; Intestine, Small; Protease Inhibitors; Sensitivity and Specificity; Swine; Trypsin Inhibitors

Year: 2005
Source title: Electrophoresis
Volume: 26
Issue: 6
Page : 1038-1045

Cited by: 7
Link: Scorpus Link
Chemicals/CAS: aprotinin, 11004-21-0, 12407-79-3, 50936-63-5, 52229-70-6, 58591-29-0, 9050-74-2, 9075-10-9, 9087-70-1; papain, 9001-73-4; proteinase inhibitor, 37205-61-1; proteinase, 9001-92-7; soybean trypsin inhibitor, 9078-38-0; trypsin, 9002-07-7; 4-methyl-coumaryl-7-amide; Chymotrypsin, EC 3.4.21.1; Coumarins; Fluorescent Dyes; Protease Inhibitors; Trypsin Inhibitors
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
ISSN: 1730835
CODEN: ELCTD
DOI: 10.1002/elps.200306142
PubMed ID: 15669004
Language of Original Document: English
Abbreviated Source Title: Electrophoresis
Document Type: Article
Source: Scopus
Authors with affiliations:

1. 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
2. Ohashi, A., Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan
3. Hirose, S., Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan
4. Katunuma, N., Tokushima Bunri University, Institute for Health Sciences, 180 Nishihamabouji, Yamashiro-cho, Tokushima City, Tokushima 770-8514, Japan

References:

1. Monard, D., (1988) Trends Neuropsin., 10, pp. 760-764
2. Nakaya, N., Nishibori, M., Kawataba, M., Saeki, K., (1996) Mol. Brain Res., 42, pp. 293-300
3. Solomon, M., Belenghi, B., Dellodonne, M., Menachem, E., Levine, A., (1999) Plant Cell, 11, pp. 431-443
4. Parson, S.L., Watson, S.A., Brown, P.D., (1997) Brit. J. Surg., 84, pp. 160-166
5. Hiemstra, P.S., (2002) Biochem. Soc. Trans., 30, pp. 116-120
6. Hanspal, J.S., Bushell, G.R., Ghosh, P., (1983) Anal. Biochem., 132, pp. 288-293
7. Michcaud, D., Faye, E., Yelle, A., (1993) Electrophoresis, 14, pp. 94-98
8. Herron, G., Michael, S., Banda, J., Clark, J., Gavrilovic, J., Werb, Z., (1986) J. Biol. Chem., 261, pp. 2814-2818
9. Stakus, P., Frank, R., Masiar, Z., Leo, J., Hawkes, S.J., (1991) Biol. Chem., 266, pp. 449-454
10. Garcia, L., Dimer, L., Norman, F., (1993) Anal. Biochem., 214, pp. 65-69
11. Pellegrini, A., Hageli, G., Fretz, D., Von Fellenberg, R., (1984) Anal. Biochem., 138, pp. 335-339
12. Pham, T.C., Konopska-Waliszkiewicz, Leluk, J., (1986) J. Biochem. Physiol. Pflanzen, 181, pp. 565-569

13. Kawataba, S., Miura, T., Morita, T., Kato, H., Furikawa, K., Iwanaga, S., Takada, K., Sakakibara, S., (1988) Eur. J. Biochem., 172, pp. 17-25
14. Iwanaga, S., Morita, T., Kato, H., Harada, T., Adachi, N., Sugo, T., Maruyama, I., Sakakibara, S., (1979) KININS-II: Biochemistry, Pathophysiology, and Clinical Aspects, pp. 147-163. , Plenum Publishing, New York
15. Bradford, M., (1976) Anal. Biochem., 72, pp. 248-254
16. Laemmli, U.K., (1970) Nature, 227, pp. 680-685
17. Yasothornsrikul, S., Hook, V., (2000) BioTechniques, 28, pp. 1166-1173
18. Bryant, J., Green, T.R., Gurusaddaiah, T., Ryan, C.A., (1976) Biochemistry, 15, pp. 3418-3424
19. Beckwilder, J., Schipper, B., Bakker, P., Bocsch, D., Jongsma, M., (2000) Eur. J. Biochem., 267, pp. 1975-1984
20. Salmela, M.T., Karialainen, M.L., Jeskanen, L., Saarialho, U., (2003) Mod. Pathol., 16, pp. 108-114
21. McKaig, B.C., McWiliam, D., Watson, S.A., Mahida, Y.R., (2003) Am. J. Pathol., 162, pp. 1355-1360
22. Weder, J., Hauner, K., Bokor, M., (1993) Electrophoresis, 14, pp. 220-226
23. Didier, B., Sylvie, D., Michelle, A., Catherine, F., Joseph, G., (1996) Biochem. J., 313, pp. 555-560
24. McManus, M., William, A., Christeller, T., Derek, W.R., (1994) Plant Physiol., 106, pp. 771-777
25. Sawada, H., Yokosawa, H., Shin-Ichi, I., (1984) J. Biol. Chem., 259, pp. 2900-2904
26. Sheau, L., Dickson, R., Chen, Y.L., (2000) J. Biol. Chem., 275, pp. 36720-36725
27. Strauss, A.W., Zimmerman, M., Boime, I., Ashe, B., Mumford, R.A., Alberets, A.W., (1979) Proc. Natl. Acad. Sci. USA, 76, pp. 4225-4229
28. Sawada, H., Yokosawa, H., Hoshi, M., Ishii, S., (1983) Experientia, 39, pp. 377-378
29. Ohashi, A., Murata, E., Yamoto, K., Majima, E., Sano, E., Le, Q.T., Katunuma, N., (2003) Biochem. Biophys. Res. Commun., 306, pp. 98-103