Cold-adapted protease enables quantitation of surface proteins in the absence of membrane trafficking

PROTOCOL FOR:

Cold-adapted protease enables quantitation of surface proteins in the absence of membrane trafficking

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BioTechniques Protocol Guide, January 2017; doi 10.2144/000114502

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Protocol Summary: We report here an improved method for analyzing protein surface expression utilizing a cold-adapted trypsin. Preservation of activity of the enzyme at 0–4°C permits modification of the protease method of surface analysis to temperatures at which trafficking of mammalian plasma-membral proteins is blocked. This is an important advantage over established trypsin-cleavage protocols. Moreover, the method is less time-consuming than surface biotinylation.

proteolytic activity of cod trypsin on ice and chymotrypsin at 34°C are comparable. (B) Hippocampal slices were treated with identical concentrations of cod trypsin on ice and with bovine chymotrypsin at 34°C for 60 min. Untreated slices served as a control. The chymotrypsin and trypsin proteolytic cleavage products of KCC2 were identified at different sizes and are denoted by an arrowhead and an arrow, respectively. There was a large decrease in the full-length KCC2 signal (denoted by block arrow) for slices treated with bovine chymotrypsin at 34°C compared with the slices treated with cod trypsin on ice. Correspondingly, the immunoreactive signal for the cleavage product was much more intense after treatment with bovine chymotrypsin at 34°C.

Protocol Schematic: Comparison of cod trypsin and mammalian chymotrypsin proteolysis of surface proteins. (A) Hippocampal slices were treated with identical concentrations of cod trypsin on ice and with bovine chymotrypsin at 34°C and on ice for 60 min and compared with the untreated control slices. Homogenization of the slices, electrophoresis, immunoblotting, and densitometry were carried out as described elsewhere (5). While there was only a slight proteolysis of GluA4 by bovine chymotrypsin on ice, the