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HPTLC for the analysis of protein digests and peptides

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Proteins play an important role in cell structures and functions. Commonly used methods for protein analysis are gel electrophoresis and mass spectrometry. Proteins are separated on polyacryl amid gel by electrophoresis, followed by tryptic digest, LC and sequence identification with mass spectrometry. Here we present HPTLC as an additional, powerful tool for the analysis of protein digests and peptides especially for peptide mapping.

The overall advantage of HPTLC is its simplicity and the high flexibility in detection. Different general stains for amino group containing compounds, such as ninhydrin or fluorescamin, are available for two dimensional peptide maps or one dimensional fingerprints of digested proteins. Additional information can be achieved with selective stains for special single amino acids or for specific modifications on amino acids.

The analysis of post- translational protein modifications such as phosphorylations is highly important in the bio analysis because reversible protein phosphorylations play a central role in functional protein regulation, especially in carcinogenesis.

Merck ProteoChrom® HPTLC Cellulose sheets and Merck ProteoChrom® HPTLC Silica gel 60 F254s plates are especially developed for fast and easy one- and two dimensional peptide separations. Appropriate mobile phases are 2-butanol/acetic acid/pyridine/water and 2-butanol/ammonia/pyridine/water.

Moreover we have developed a procedure on ProteoChrom® HPTLC Cellulose sheets for selective detection of phosphorylations after tryptic digest of proteins based on ProQ® Diamond Phosphoprotein Blot Stain Kit (Invitrogen, Karlsruhe, Germany) followed by a general staining of all peptides.