

iST: Sample preparation for high throughput clinical proteomics

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Sample preparation workflows are a crucial part of routine mass spectrometry (MS) based proteomics measurements. Complex workflows, extensive sample fractionation and proteolytic digestion are highly time consuming and restrict the overall technical reproducibility limiting the overall applicability of MS-based proteomics for clinical applications. The overall accuracy and robustness of the MS platform is also strongly affected by sample quality reasoning for high quality proteomic samples. Here we present the straightforward and robust in-StageTip (iST) method for streamlined sample processing of complete proteomes (Fig 1).

The novel iST method is a 3-step procedure performed in a single, enclosed volume which thereby circumvents the likelihood of contamination and sample loss. Due to the straightforward nature, the method can readily be performed in a 96-well format. The method is highly compatible with established and novel StageTip based pre-fractionation methods and thereby allows in-depth analysis of complex proteomic samples. Applying the procedure to the well-studied cancer cell line HeLa allowed us to estimate protein copy-numbers of 9,667 proteins. The results demonstrated excellent reproducibility ($R^2 = 0.97$) in quadruplicate measurements reflecting the overall strength of the method. The in-StageTip method opens up opportunities for high-throughput clinical applications enabling exceptional sample quality at low cost and effort. The iST method is simple and generally applicable even to large-scale clinical applications.

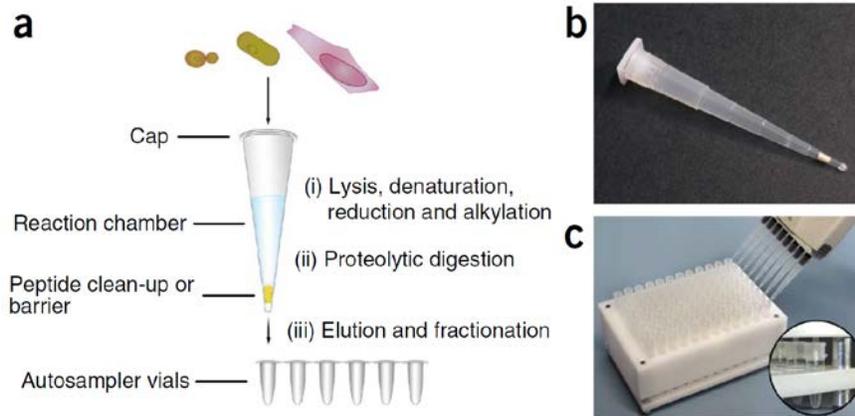


Figure 1: Minimal sample-preparation procedure for rapid and robust processing of clinically relevant samples. (a) The iST sample preparation workflow. Samples are lysed, proteins denatured and digested and peptides are cleaned-up with minimal effort. (b) Enclosed sample preparation devices avoid sample contamination and guarantee little sample loss. (c) Multiplexed sample-preparation in a 96-well format enable high-throughput applications. (Kulak N.A. et al, Nat Methods. 2014, doi: 10.1038/nmeth.2834)