

## **Rapid Release of N-Linked Glycans from Glycoproteins by Pressure-Cycling Technology**

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The standard, well-established sample preparation protocol to release N-linked glycans from glycoproteins for downstream analysis requires relatively long deglycosylation times (from several hours to overnight) and relatively high endoglycosidase concentration (from 1:250 to 1:500 enzyme:substrate molar ratio). In this paper, we significantly improve this standard protocol by the use of pressure-cycling technology (PCT) to increase the speed and decrease the relative amount of PNGase F during the release of N-linked glycans from denatured glycoproteins. With the application of pressure cycling from atmospheric to as high as 30 kpsi, >95% release of the asparagine-linked glycans from bovine ribonuclease B, human transferrin, and polyclonal human immunoglobulin was rapidly achieved in a few minutes using as low as 1:2500 enzyme:substrate molar ratio. The deglycosylation rate was first examined by SDS-PAGE at the protein level. The released glycans were then quantitated by capillary electrophoresis with laser induced fluorescence detection (CE-LIF). This new sample preparation protocol readily supports large-scale glycan analysis of biopharmaceuticals with rapid deglycosylation times.

The full text can be downloaded from:

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