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Deglycosylation of N-glycosylated proteins using PNGase F

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ABSTRACT

The described protocol was used to confirm that NADPH-cytochrome P450 reductase from *Helianthus annuus* was *N*-glycosylated when recombinantly expressed in *Pichia pastoris* (*Komagataella phaffii*).

MATERIALS

PNGase F from Elizabethkingia meningoseptica **Sigma Aldrich Catalog #P7367-50UN**

1 12,5 µl of yeast lysate containing sufficient amount of target protein for detecting by Western analysis was pipetted into a tube. Two samples were prepared in parallel, one for negative control without *N*-glycosidase and the other with PNGase F.

2 0,5 µl of 10% sodium dodecyl sulfate (SDS) and 1 µl of 1M dithiothreitol (DTT) were added.

3 The mixtures were incubated at 95 °C for 5 min.

4 The mixtures were cooled to room temperature after which 2 µl of 0,5 M Tris-HCl, pH 8 and 2 µl of 10% Triton X-100 were added.

5 2 µl of PNGase F (500 U/ml) was added to one of the samples. Instead of *N*-glycosidase, water was added to the negative control sample.

6 The samples were incubated overnight at 37 °C.

7 SDS-PAGE sample buffer was added and the samples were heated for 5 min at 99 °C. SDS-PAGE and Western analysis were carried out to see whether the molecular weight of the target protein had decreased.