Jan 09, 2023

Deglycosylation of N-glycosylated proteins using PNGase F

DOI
dx.doi.org/10.17504/protocols.io.x54v9dr84g3e/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.x54v9dr84g3e/v1

Protocol Citation: Kaia Kukk 2023. Deglycosylation of N-glycosylated proteins using PNGase F . **protocols.io https://dx.doi.org/10.17504/protocols.io.x54v9dr84g3e/v1**

Manuscript citation:
Kukk, K. (2023) Identification, characterisation and recombinant expression of flavonoid 3′,5′-hydroxylases and cytochrome P450 reductases from *Vaccinium* species. *bioRxiv* 2023.01.09.523147; doi: https://doi.org/10.1101/2023.01.09.523147

Protocol status: Working
We use this protocol and it's working

Created: December 09, 2022

Last Modified: January 09, 2023

Protocol Integer ID: 73770

Keywords: cytochrome p450 reductase from helianthus annuus, glycosylated protein, cytochrome p450 reductase, deglycosylation, pichia pastori, helianthus annuus

Funders Acknowledgements:
European Regional Development Fund
Grant ID: Grant ID: 1.1.1.2/VIAA/2/18/286

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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P7367-50UN

Troubleshooting

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.