

PNGase F Protocol

Overview

Protocols.io also provides an interactive version of this protocol where you can discover and share optimization with the research community. This is available for both [PNGase F Protocol, Denaturing Conditions](#) and [PNGase F Protocol, Non-Denaturing Conditions](#).

Reactions may be scaled-up linearly to accommodate larger amounts of glycoprotein and larger reaction volumes. Optimal incubation times may vary for particular substrates. Typical reaction conditions are as follows:

Protocol

1. Denaturing Reaction Conditions:

1. Combine 1-20 µg of glycoprotein, 1 µl of Glycoprotein Denaturing Buffer (10X) and H₂O (if necessary) to make a 10 µl total reaction volume.
2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3. Chill denatured glycoprotein on ice and centrifuge 10 seconds.
4. Make a total reaction volume of 20 µl by adding 2 µl GlycoBuffer 2 (10X), 2 µl 10% NP-40 and 6 µl H₂O.
PNGase F is inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture under denaturing conditions. Failure to include NP-40 into the denaturing protocol will result in loss of enzymatic activity.
5. Add 1 µl PNGase F, mix gently.
6. Incubate reaction at 37°C for 1 hour.
7. Analyze by method of choice

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

2. Non-Denaturing Reaction Conditions:

When deglycosylating a native glycoprotein it is recommended that an aliquot of the glycoprotein is subjected to the denaturing protocol to provide a positive control for the fully deglycosylated protein. The non-denatured reaction can then be compared to the denatured reaction to determine the extent of reaction completion.

1. Combine 1-20 µg of glycoprotein, 2 µl of GlycoBuffer 2 (10X) and H₂O (if necessary) to make a 20 µl total reaction volume.
2. Add 2-5 µl PNGase F, mix gently.
3. Incubate reaction at 37°C for 4 - 24 hours.

Note: To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

4. Analyze by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

Notes

- If using [P0704/P0708](#), we recommend limiting PNGase F to 1/10 (or less) of the total reaction volume to keep the final glycerol concentration equal to (or less than) 5%.
- For unit conversion between different suppliers, please reference the [Glycobiology Unit Conversion Chart](#) page.