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 H_2O (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 H_2O.
    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 H_2O (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.