PNGase F Protocol, Non-Denaturing Conditions

New England Biolabs

Abstract

This is a generic PNGase F protocol with non-denaturing reaction conditions. It is appropriate for both P0704 and P0708. Typical reaction conditions are below.

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Guidelines

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.

Before start

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.

Protocol

Step 1.
Combine the following (for a 20 µl total reaction volume):

PROTOCOL

. PNGase F Non-Denaturing Mixture

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NOTES
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Reactions may be scaled-up linearly to accommodate larger amounts of glycoprotein and larger reaction volumes.
Step 1.1.
Glycoprotein, **1-20 µg**

Step 1.2.

10X G7 Reaction Buffer, **2 µl**

AMOUNT

2 µl Additional info:

Step 1.3.

H2O (if necessary) to make a **20 µl** total reaction volume.

Step 2.

Add 2-5 µl PNGase F

NOTES

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For unit conversion between different suppliers, please reference the Glycobiology Unit Conversion Chart page.

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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%.

Step 3.

Mix gently

Step 4.

Incubate reaction at 37°C for 4 - 24 hours

NOTES

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Optimal incubation times may vary for particular substrates.

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

Step 5.

Analyze by method of choice

NOTES

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