PNGase F Protocol, Denaturing Conditions

New England Biolabs

Abstract

This is a generic PNGase F protocol with denaturing reaction conditions. It is appropriate for both P0704 and P0708.

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Before start

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 below.

Protocol

Step 1.
Combine the following (for a **10 µl** total reaction volume):

PROTOCOL

. PNGase F Mixture 1

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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 Glycoprotein Denaturing Buffer, 1 µl

AMOUNT
1 µl Additional info:

Step 1.3.
H2O (if necessary) to make a **10 µl** total reaction volume

Step 2.
Denature glycoprotein by heating reaction at 95°C for 5 minutes.

DURATION
00:05:00

Step 3.
Chill denatured glycoprotein on ice

Step 4.
Centrifuge 10 seconds

DURATION
00:00:10

Step 5.
Add the following (bringing total reaction volume to **20 µl**):

PROTOCOL

. PNGase F Mixture 2

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NOTES
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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.

Step 5.1.
10X G7 Reaction Buffer, **2 µl**

AMOUNT
2 µl Additional info:

Step 5.2.
10% NP40, **2 µl**

AMOUNT
2 µl Additional info:

Step 5.3.
H2O, **6 µl**

AMOUNT
6 µl Additional info:

Step 6.
Add PNGase F, **1 µl**

AMOUNT
1 µl Additional info:

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

Step 7.
Mix gently

Step 8.
Incubate reaction at 37°C for 1 hour

NOTES

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Optimal incubation times may vary for particular substrates.

Step 9.

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.