ab308242 – PNGase F Deglycosylation Kit

For the quick deglycosylation of glycoproteins using PNGase F under optimized conditions.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

Store the kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
PNGase F Reaction Buffer (5X)	1 ml	-20°C
Denaturation Buffer (10X)	0.5 ml	-20°C
Detergent Solution (10X)	0.5 ml	-20°C
Recombinant PNGase F	200 µl	-20°C
Glycoprotein Control	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Equipment to run SDS-PAGE
- Staining & detaining reagents

Reagent Preparation

PNGase F Reaction Buffer (5X), Denaturation Buffer (10X) & Detergent Solution (10X): Warm to room temperature (RT) before use. Store at -20 °C.

Recombinant PNGase F: Ready to use. Divide into aliquots and store at -20 °C. Keep on ice, while in use. Avoid freeze and thaw cycles. Use within two months.

Glycoprotein Control: Reconstitute the vial in 40 µl of distilled water to prepare Glycoprotein Control solution. Divide into aliquots & store at -20 °C.

Deglycosylation Assay Protocol:

1. Preparation of Glycoprotein Sample:

Prepare Glycoprotein Sample in 1X PNGase F Reaction Buffer.

2. Preparation of Deglycosylation Mix:

For each Glycoprotein Sample, prepare 45 µl of Deglycosylation Mix containing:

	Deglycosylation Mix
PNGase F Reaction Buffer (5X)	10 µl
Denaturation Buffer (10X)	5 µl
Glycoprotein Sample	50-500 µg (in 30 µl)
Adjust the volume to (with water)	45 µl

3. Mix well and heat at 100 °C for 10 min.
4. Spin briefly and cool down the solution
5. Add 5 µl of 10X Detergent Solution and mix well.
6. Add 2 µl of Recombinant PNGase F to the above solution. Mix well and incubate at 37 °C for 2 hr.
7. Stop the enzymatic reaction by putting the vial on ice. This is the deglycosylated sample.

Δ Note:

a. *The amount of Recombinant PNGase F needed for the complete deglycosylation might vary depending on the Glycoprotein Sample(s). We recommend performing a pilot experiment to determine the ratio of glycoprotein sample to PNGase F and the incubation time.*
b. *Dilute the Recombinant PNGase F in 1X PNGase F Reaction Buffer, if required.*

8. Analyze the deglycosylated sample on a gradient SDS-PAGE, while keeping the remaining reaction mix on ice. The remaining reaction mix can be lyophilized or frozen for downstream applications including mass spectrophotometric analysis of the deglycosylated protein etc.

Glycoprotein Control (optional):

As a Positive Control, use 10 µl of Glycoprotein Control solution instead of Glycoprotein Sample in the above Deglycosylation Mix and follow step 2 to step 6, but reduce the volume of the reagents accordingly.

SDS-PAGE Analysis of Deglycosylated Sample(s)

Add 5 µl of 3X SDS-loading buffer to 10 µl of deglycosylated sample(s) (from step 7) and heat for 5 min at 100 °C. Cool down the sample(s) and load 5-10 µl on a gradient SDS-PAGE. Load equal amount of non-deglycosylated glycoprotein sample as the Negative Control.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)