SmartEnzymes™

GENOVIS

PNGase F

Automation

STORE AT

-20°C

FOR RESEARCH USE ONLY

Instructions for Use

PNGase F Automation 96 × 5 units (G1-PFA-005)
Process 96 × 5 µg glycoprotein

PNGase F Automation 96 × 50 units (G1-PFA-050)
Process 96 × 50 µg glycoprotein

DOWNLOAD INSTRUCTIONS FOR USE

www.genovis.com/ifu-G1-PFA

www.genovis.com

Lyophilized Enzyme in 96-well Plates for Automated Hydrolysis of N-glycans on Glycoproteins

PNGase F (Peptide N-glycosidase F) is a glycoamidase hydrolyzing the amide bond between the polypeptide asparagine and the innermost GlcNAc of all mammalian asparagine-linked complex, hybrid, or high-mannose oligosaccharides. PNGase F Automation contains the PNGase F enzyme lyophilized in an automation-friendly 96-well plate format.

During the reaction, the asparagine residue from which the glycan is removed is deamidated to aspartic acid. The released oligosaccharide is left intact and can be used for further analysis. Removal of N-glycans is widely used in sample preparation for MS analysis – to reduce the protein heterogeneity and enable released glycan analysis – and to study the functional role of the N-glycan.

PNGase F is expressed in *E. coli* from a recombinant gene derived from *Elizabethkingia meningoseptica*. The enzyme contains a His-tag and has a molecular weight of 36 kDa.

UNIT DEFINITION

One unit PNGase F removes N-glycans from ≥ 95% of 1 µg etanercept when incubated in TBS buffer (50 mM Tris-HCl, 150 mM NaCl) pH 8.6 at 37°C for 1 h.

CONTENT AND STORAGE

PNGase F Automation is supplied lyophilized in 50 mM HEPES buffer pH 7.5, with no preservatives added. The enzyme is shipped at ambient temperature, and should be stored at -20°C upon arrival.

PNGase F Automation is for R&D use only.

QUALITY CONTROL

PNGase F Automation is tested to meet the specifications and lot-to-lot consistency.

YOU MIGHT ALSO BE INTERESTED IN

PNGase F Lyophilized
Lyophilized enzyme for hydrolysis of N-glycans

PNGase F Immobilized
Immobilized enzyme for hydrolysis of N-glycans on glycoproteins in spin columns

OmniGLYZOR™
Hydrolysis of N- and mucin-type O-glycans

OpeRATOR®
O-glycan-specific protein digestion

Preparations

Important Information

- Quickly spin down the plate before use.
- The foil seal can be pierced with a pipette tip or removed from the plate before addition of the glycoprotein solution.
- Optimization of substrate concentration, incubation time and incubation temperature may be needed depending on the substrate. Some glycoproteins require denaturing and/or reducing reaction conditions to reach complete deglycosylation.

Additional Materials Required

- Reaction buffer: PBS, pH 7.4.[1]

Sample Preparation

Prepare the glycoprotein in the reaction buffer (minimum 10 µl/well, maximum 150 µl/well). Low substrate concentrations will require longer incubation times.

1. PNGase F is active at pH 6.0-9.0 and over a wide range of ionic strengths (0-1000 mM NaCl). Optimization may be required if buffers other than the recommended are used.

Automated Hydrolysis of N-glycans on Glycoproteins

1. Add glycoprotein

1.1 Add 1 µg glycoprotein / 1 unit PNGase F. Mix by carefully pipetting up and down.

2. Digestion

2.1 Seal the plate[2] and incubate for 1 hour to overnight at 37°C.

2. Seal the plate with a sealing mat, adhesive seal, Parafilm® or similar.

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