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Product Information

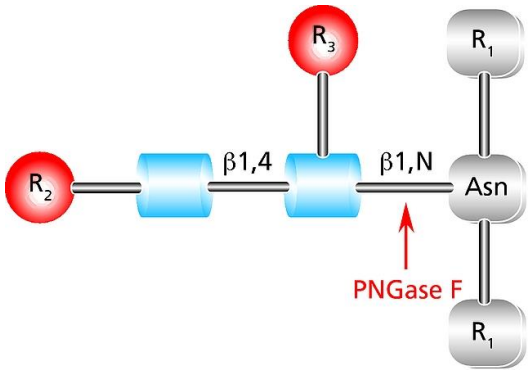
PNGase Fast Kit
PNGase F from *Elizabethkingia meningoseptica* recombinant, expressed in *E. coli*

Catalog Number **EMS0001**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

CAS RN 83534-39-8
EC 3.5.1.52
Synonyms: N-Glycosidase F, Peptide N-glycosidase

Product Description
PNGase F (Peptide N-Glycosidase F) cleaves asparagine-linked high mannose as well as hybrid and complex oligosaccharides from glycoproteins. It deaminates the asparagine to aspartic acid, but leaves the oligosaccharide intact. PNGase F will not remove oligosaccharides containing α(1→3)-linked core fucose, commonly found in plant glycoproteins. A tripeptide with the oligosaccharide-linked asparagine as the central residue is the minimal substrate for PNGase F.



Molecular mass: 36 kDa
pH range: 6–10
Optimal pH: 8.6.

Components
Sufficient reagents for 50 × 100 µg reactions

- Denaturing Buffer, 5× Reaction Buffer 500 µL
  Proprietary detergent solution in buffer
  Catalog Number EMS0002

- PNGase F Enzyme Solution 50 µL
  Supplied in a solution of 20 mM Tris-HCl, pH 7.5, containing 1 mM EDTA and 50 mM NaCl
  Catalog Number EMS0003

Reagents and Equipment Required but Not Provided.
- 2-Mercaptoethanol (βME, Catalog Number M6250 or equivalent)
- 30 kDa MWCO Centrifugal Filtration Units, 0.5 mL (Catalog Number MRCF0R030, UFC5030, or equivalent). 10 kDa filters will also work, but spin time must be increased.
- 50 mM ammonium bicarbonate solution

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
The enzyme and reaction buffer are ready to use.

Storage/Stability
The kit should be stored at 2–8 °C. Components have been shown to be stable for at least 2 years if stored properly. The detergent solution may crystallize at 2–8 °C, but can easily be resolubilized by heating at 70 °C for 5 minutes and vortexing. Repeat if necessary.

Procedures

Sample preparation and handling

For best results, it is recommended to start with protein concentrations of 5–10 mg/mL in a low salt buffer. NaCl concentrations of greater than 250 mM can cause proteins to aggregate and precipitate during denaturation.

The following procedure is scaled for 10 µL of a protein solution, which should correspond to 50–100 µg of protein mass. Volumes of reagents can be scaled appropriately if larger or smaller sample inputs are desired. If concentration of the sample is necessary, it is recommended to desalt, dry down, and reconstitute the protein in water to achieve the desired concentration.

The detergents in the Denaturing Buffer can interfere with subsequent glycan labeling and analysis. These can be efficiently removed using centrifugal filtration units noted in the optional reagents section. For analysis of deglycosylated proteins, detergent removal may not be necessary. Procedure, Step 2 can be omitted.

Note: Some proteins are not amenable to fast deglycosylation techniques. It is always best to compare the results of a fast digest to a traditional overnight digest or the established protocol in use.

Deglycosylation Procedure

1. Denature glycoprotein
   a. Transfer 10 µL of sample solution (50–100 µg of protein) to a small tube.
   b. Add 2.5 µL of Denaturing Buffer (Catalog Number EMS0002).
   c. Dilute βME 100-fold in water and add 0.5 µL to the reaction mix to make ~5 mM in βME.
   d. Mix well.
   e. Incubate at 70 °C for 10 to 15 minutes to denature. For proteins which have shown a tendency to aggregate or precipitate when heated, a lower denaturing temperature may be used. For example, 50 °C has been shown to be effective for some proteins, but in general deglycosylation is most efficient after denaturing at 70 °C.
2. Detergent removal
   a. Transfer the denatured protein solution to the filter cup of a 30 kDa centrifugal filtration unit.
   b. Centrifuge at 10,000–14,000 × *g* for 5 minutes.
   c. Add 100 µL of 50 mM ammonium bicarbonate.
   d. Centrifuge at 10,000–14,000 × *g* for 5 minutes.
   e. Transfer the filter cup to a clean tube.
   f. Add 50 µL of 50 mM ammonium bicarbonate to the filter cup and transfer the filter unit to 37 °C.
3. Deglycosylation
   a. Allow to equilibrate at 37 °C for 5 minutes.
   b. Add 1 µL of PNGase F Enzyme Solution (Catalog Number EMS0003).
   c. Mix well.
   d. Incubate at 37 °C for 10–20 minutes.
   e. The majority of glycans will be released in 10 minutes.
   f. More difficult glycans may require longer incubations.
4. Recover the glycans by centrifugation.
   a. Centrifuge at 10,000–14,000 × *g* for 5 minutes.
   b. Add 50 µL of 50 mM ammonium bicarbonate to the filter cup and centrifuge again.
   c. The glycans will be in the flow-through in the lower part of the centrifugal unit.
   d. Label as desired for analysis.
5. Deglycosylated protein can be recovered from the top of the filtration unit.
   a. Invert the filter insert into a clean tube.
   b. Centrifuge at 10,000–14,000 × *g* for 5 minutes.
   c. Rinsing the filtration unit with ammonium bicarbonate can improve recovery.

JK,JAB,MAM 03/18-1