

## Description

Peptide N-glycosidase F (PNGase F) is an endoglycosidase from *Flavobacterium meningosepticum* that is recombinantly expressed in *E. coli*. The enzyme cleaves the  $\beta$ -aspartylglucosamine bond between the N-Acetylglucosamine (GlcNAc) and asparagine linkage of N-linked oligosaccharides in glycoproteins (1).

1. Maley, F. *et al.* (1989) Anal. Biochem. 180:195.

## What's Included

PNGase F Enzyme	40 $\mu$ L
10X Deglycosylation Reaction Buffer	1 mL
10X Denaturing Solution	1 mL
15% NP40	1 mL
N-linked glycobiology standard	40 $\mu$ L

PNGase F is stored in 20 mM Tris - pH 7.5, 50 mM NaCl, 0.5 mM EDTA.

N-linked glycobiology standard (Ovalbumin Product # FSB1001)

**Storage:** Store all components at 4°C. Kit components are stable for one year.

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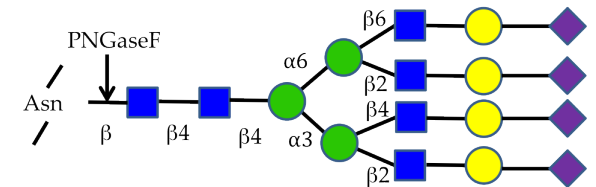
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N-LINKED DEGLYCOSYLATION KIT



**CATALOG NUMBER**  
**FSB3003**

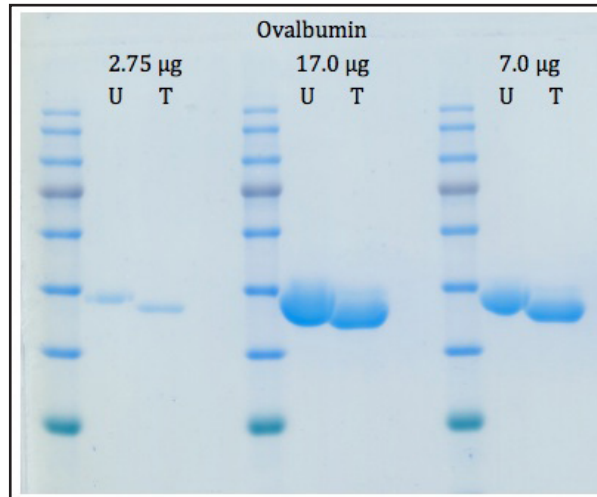
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## Reaction Protocol (Denaturation Conditions)

1. Add 1  $\mu\text{L}$  of 10X Denaturing Solution (5% SDS, 0.4 mM DTT) to 9  $\mu\text{L}$  of substrate solution (containing 1-20  $\mu\text{g}$  of glycoprotein of interest)
2. Incubate substrate solution from step 1 for 5 minutes at 99°C then place back on ice.
3. Add 2  $\mu\text{L}$  of 15% NP-40 solution, 2  $\mu\text{L}$  of 10X Deglycosylation Reaction Buffer (0.5 M sodium phosphate – pH 7.5), 4  $\mu\text{L}$   $\text{H}_2\text{O}$ , and 2  $\mu\text{L}$  PNGase F to denatured substrate. Include a control reaction by replacing the PNGase F with  $\text{H}_2\text{O}$ .
4. Incubate reaction for 60 minutes at 37°C.
5. Analyze the reaction by running treated and untreated samples in separate lanes on SDS-PAGE gel. Proteins that have been deglycosylated will have increased mobility due to reduction in molecular weight. (See Figure 1)



**Figure 1: SDS-PAGE analysis of ovalbumin deglycosylation by PNGase F at the indicated concentrations.** Untreated (U) and treated (T) ovalbumin samples are shown adjacent to a low molecular weight standard.

## Additional Information

**Remember:** PNGase F is inhibited by SDS. Therefore, the addition of the NP-40 is critical for efficient enzyme production under denaturation conditions.

**For analysis under non-denaturation conditions** - Remove 10X Denaturing Solution. However, longer incubation times and more enzyme may be required.

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