



## Fast PNGase F (Glycerol-free)

### Product description

PNGase F is an amidohydrolase that can cleave high-mannose, hybrid, and complex oligosaccharides linked by asparagine, specifically removing N-linked glycans. However, the conventional PNGase F enzymatic reaction often takes several hours to release antibody N-glycans. Due to the preferential release of glycans, incomplete deglycosylation might result in biased outcomes, with the obtained glycan distribution failing to represent the true composition of therapeutic antibodies. Therefore, rapidly obtaining an accurate N-glycan profile is crucial for glycosylation monitoring during the production of antibodies and antibody-fusion proteins used in biotherapeutics.

Yeasen Fast PNGase F (Glycerol-free) is an optimized recombinant reagent capable of thoroughly and rapidly deglycosylating antibodies, immunoglobulins, fusion proteins, and other glycoproteins within minutes. This enzyme can swiftly and non-selectively remove all N-glycans, enabling direct downstream chromatography or mass spectrometry analysis. Yeasen Fast PNGase F (Glycerol-free) simplifies the experimental procedure while reducing experimental time without compromising sensitivity and reproducibility.

Additionally, Yeasen also provides conventional PNGase F (Cat#20407ES, specific activity: 100,000 U/mL), and other types of glycosidases such as Endo H (Cat#20414ES) and Endo S (Cat#20413ES).

### Components

Components No.	Name	20406ES20	20406ES50
20406-A	Fast PNGase F	20 $\mu$ L	50 $\mu$ L
20406-B	Fast PNGase F Buffer (5 $\times$ )	80 $\mu$ L	200 $\mu$ L

### Properties

English synonym	Fast PNGase F (Glycerol-free)
Source	Yeast recombinant expression
Storage buffer	20 mM Tris-HCl pH 7.5, 50 mM NaCl, 5 mM EDTA

### Storage

The product can be stored at 2 ~ 8°C for one year, **do not freeze**.

### Instructions

1. One-step Protocol:

1) Combine up to 100  $\mu$ g of antibody and H<sub>2</sub>O to a volume of 16  $\mu$ L.



- 2) Add 4  $\mu\text{L}$  of Fast PNGase F Buffer (5X) to make a 20  $\mu\text{L}$  total reaction volume.
- 3) Add 1  $\mu\text{L}$  of Fast PNGase F.
- 4) Incubate 10 minutes at 50°C.

## 2. Two-step Protocol:

Some antibodies (i.e. Fab N-glycans) require a preheating step for efficient deglycosylation.

- 1) Combine up to 100  $\mu\text{g}$  of antibody and  $\text{H}_2\text{O}$  to a volume of 16  $\mu\text{L}$ .
- 2) Add 4  $\mu\text{L}$  of Fast PNGase F Buffer (5X) to make a 20  $\mu\text{L}$  total reaction volume.
- 3) Incubate at 80°C for 2 minutes, cool down.
- 4) Add 1  $\mu\text{L}$  of Fast PNGase F.
- 5) Incubate 10 minutes at 50°C.

## Notes

1. To achieve optimal heat transfer, use a 0.2 mL PCR tube or a 1.5 mL thin-walled centrifuge tube. Incubation can be performed using a thermal cycler with a heated lid or a metal bath.
2. It is recommended to perform a two-step enzymatic digestion to ensure higher enzymatic efficiency.
3. The storage system of the antibody or antibody-fusion protein sample must be compatible with the activity of Fast PNGase F. Since SDS inhibits the enzymatic activity of Fast PNGase F, the storage system should not contain SDS; commonly used stabilizers such as Tween, Triton X-100, NP-40, octyl glucoside, and non-detergent sulfobetaine, as well as small amounts of organic solvents, will affect the optimal rapid deglycosylation efficiency.
4. Treating under 75°C conditions for 10 minutes can inactivate Fast PNGase F.
5. Please wear the necessary PPE, such lab coat and gloves, to ensure your health and safety!
6. For research use only!