**APX267Ge01 100μg**
**Active Peptide-N4-N-Acetyl-Beta-D-Glucosaminyl Asparagine Amidase F (PNGaseF)**
**Organism Species:** ***Pan-species (General)***
***Instruction manual***

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

## [ PROPERTIES ]

**Source:** Prokaryotic expression.
**Host:** *E. coli*
**Residues:** Ala41~Asn354
**Tags:** N-terminal His-tag
**Purity:** >90%
**Endotoxin Level:** <1.0EU per 1μg (determined by the LAL method).
**Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
**Applications:** Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
**Predicted isoelectric point:** 8.0
**Predicted Molecular Mass:** 38.5kDa
**Accurate Molecular Mass:** 40kDa as determined by SDS-PAGE reducing conditions.

## [ USAGE ]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

## [ STORAGE AND STABILITY ]

**Storage:** Avoid repeated freeze/thaw cycles.
Store at 2-8°C for one month.
Aliquot and store at -80°C for 12 months.

**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

## [ SEQUENCE ]

APADNTVNIKTFDKVKNAFGDGLSQSAEGTFTFPADVTTVKTIKMFIKNECPNKTCDEWDRYANVYVKNKTTGEWYEIGRFITPYWVG
TEKLPRGLEIDVTDFKSLLSGNTELKIYTETWLAKGREYSVDFDIVYGTPDYKYSAVVPVIQYNKSSIDGVPYGKAHTLGLKKNIQLP
TNTEKAYLRTTISGWGHAKPYDAGSRGCAEWCFRTHTIAINNANTFQHQLGALGCSANPINNQSPGIWAPDRAGWCPGMAVPTRIDVL
NNSLTGSTFSYEYKFQSWTNNGTNGDAFYAISSFVIAKSNTPISAPVVTN

## [ ACTIVITY ]

PNGase F (Peptide-N-glycosidase F) is a kind of enzymes for the deglycosylation of glycoproteins. The enzyme releases asparagine-linked oligosaccharides from glycoproteins and glycopeptides by hydrolyzing the amide of the asparagine (Asn) side chain. Thus, the activity of recombinant PNGase F measured by deglycosylating Interferon Gamma (IFNg) under denatured conditions. Prepare 10×denaturing buffer(5% SDS,400mM DTT), dilute IFNg to 1 μg/μl by 1×denaturing buffer,then heat the solution to 100℃for 10 minutes to denature the glycoprotein. Cool to room temperature and microcentrifuge briefly. Dobule dilution of recombinant PNGase F by assay buffer(50mmol/L Tris(pH7.4),1% NP-40), add 10 μl denatured IFNg to 10 μl different concentration of recombinant PNGase F, incubate reaction mixture at 37℃ for 1 hour. Stop the reaction by heating to 100℃ for 5 minutes, assess deglycosylation by SDS-PAGE. In the 10 μl reaction system, the amount of PNGase F needed to remove more than 95% carbohydrates from 10 μg denatured IFNg in 1 hour at 37℃ was defined as a unit. In this procedure, one unit is equal to 2.5ng recombinant PNGase F . The results are shown in Figure 1.

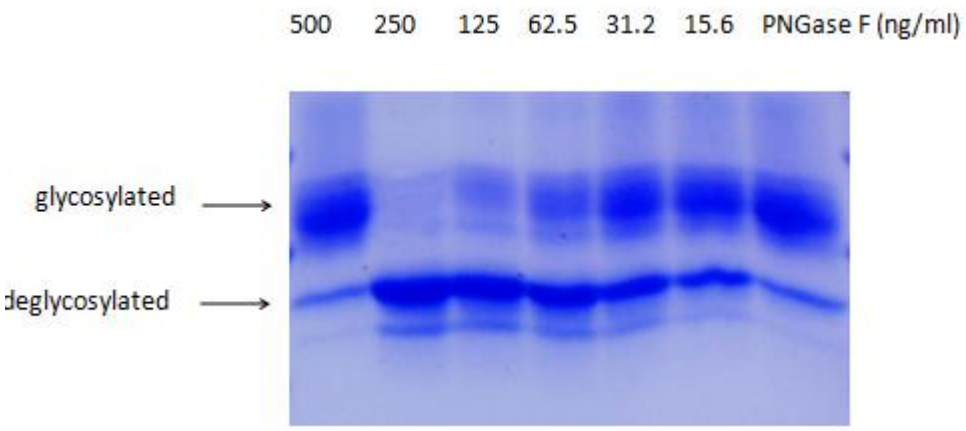


**Figure 1. The deglycosylation of IFNg detect by SDS-PAGE**

## [ IDENTIFICATION ]

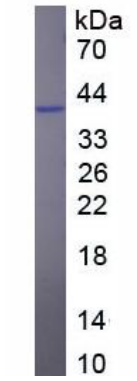


**Figure 2. SDS-PAGE**

**Sample: Active recombinant PNGaseF, General**

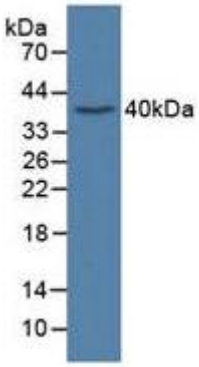


**Figure 3. Western Blot**

**Sample: Recombinant PNGaseF, General;**

**Antibody: Rabbit Anti-General PNGaseF Ab (PAX267Ge01)**

## [ IMPORTANT NOTE ]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.