Recombinant *F. meningosepticum* PNGase F

Catalog Number: 9109-GH

DESCRIPTION

Source	*E. coli*-derived *f. meningosepticum* PNGase F protein Ala41-Asn354 with N-terminal Met and 6-His tag Accession # P21163
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	34 kDa, reducing conditions
Activity	Measured by its ability to deglycosylate ribonuclease B under denatured conditions. >50% ribonuclease B (10 µg) is deglycosylated by 2.5 ng rFmPNGase F within 30 minutes, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M Tris, pH 7.5
- Denaturing Buffer (10X): 5% SDS, 0.8 M β-Mercaptoethanol
- Recombinant *F. meningosepticum* PNGase F (rFmPNGase) (Catalog # 9109-GH)
- Ribonuclease B, from bovine pancreas (RNase B) (Sigma, Catalog # R7884), 2.5 mg/mL stock in 25 mM Tris, pH 7.5
- 10% Triton® X-100 (Amresco, Catalog # M236)
- Reducing SDS-PAGE Sample Buffer
- SDS-PAGE or Western Blot

Assay

1. Dilute Denaturing Buffer to 5X in deionized water.
2. Create a Substrate Mixture containing 0.8 mg/mL RNase B and 1X Denaturing Buffer in deionized water.
3. Heat Substrate Mixture at 100 °C for 10 minutes. Cool to room temperature and microcentrifuge briefly.
4. Add 10% Triton® X-100 to a final concentration of 1.67%.
5. Dilute r*Fm*PNGase F to 0.167 ng/µL in Assay Buffer.
6. Combine 15 µL of Substrate Mixture and 15 µL 0.167 ng/µL r*Fm*PNGase F. Include a control containing 15 µL of Substrate Mixture and 15 µL of Assay Buffer.
7. Incubate reaction mixture at 37 °C for 30 minutes.
8. Combine equal volumes of incubated reaction mixture and reducing SDS-PAGE sample buffer and boil samples at 100 °C for 3-5 minutes.
9. Load 15 µL (2.5 µg RNase B) per lane on a 4-20% SDS-PAGE gel.
10. Stain gel and analyze for percent deglycosylation using densitometry.

Final Assay Conditions

Per Reaction:

- rFmPNGase F: 2.5 ng
- RNase B: 10 µg

PREPARATION AND STORAGE

Shipping

The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage

Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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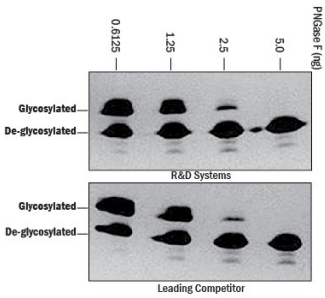
Recombinant *F. meningosepticum* PNGase F

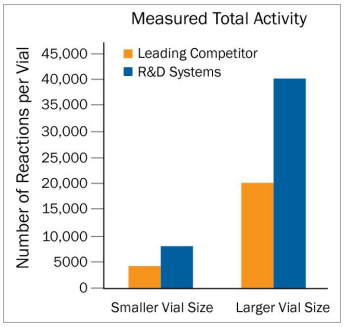
Catalog Number: 9109-GH

Enzyme Activity



Recombinant *F. meningosepticum* PNGase F (Catalog # 9109-GH) from R&D Systems and a leading competitor are able to deglycosylate 10 µg of RNase B at 37 °C in one hour. The *E. coli*-produced enzyme from R&D Systems offers a better value than the competition

Bioactivity



Total activity per vial of Recombinant F. meningosepticum PNGase F (Catalog # 9109-GH) compared to the leading competitor. R&D Systems® PNGase F gives you 2x more enzyme at a comparable price.

BACKGROUND

PNGase F, peptide N-glycosidase F from *Flavobacterium meningosepticum*, catalyzes the hydrolysis of asparagine-linked high mannose, as well as hybrid and complex oligosaccharides from glycoproteins (1). Unlike glycosidases that hydrolyze glycosidic bonds, PNGase F is an amidase that cleaves the beta-aspartylglucosamine bond between the innermost GlcNAc of N-glycans and asparagine residues of glycoproteins (2). The enzyme is highly active on various N-glycans except those with the innermost GlcNAc modified with alpha 1-3-linked core fucose, which is commonly found on plant glycoproteins (3). Cleavage with PNGase F will convert the asparagine residue to an aspartic residue, allowing identification of the glycosylation site by mass spectrometry (4). This purified enzyme is compatible with glycan analysis using mass spectrometry.

References:

1. Elder, J.H. and Alexander, S. (1982) Proc. Natl. Acad. Sci. USA **79**:4540.
2. Maley, F. *et al*. (1989) Anal. Biochem. **180**:195.
3. Tarentino, A.L. and Plummer, T.H. (1994) Methods Enzymol **230**:44.
4. Zhang, H. *et al*. (2003) Nat. Biotechnol. **21**:660.

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