Recombinant *F. meningosepticum* PNGase F

Catalog Number: 7695-GH

DESCRIPTION

Source	*E. coli*-derived Ala41-Asn354 with N-terminal Met and 6-His tag Accession # AAA85323
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	33-35 kDa, reducing conditions
Activity	Measured by its ability to deglycosylate ribonuclease B under denatured conditions. >50% ribonuclease B (10 µg) is deglycosylated by 10 ng of r*Fm*PNGase F within 30 minutes, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
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 (r*Fm*PNGaseF) (Catalog # 7695-GH)
- Ribonuclease B, from bovine pancreas (RNase B) (Sigma, Catalog # R7884), 2.5 mg/mL stock in 25 mM Tris, pH 7.5
- 10% (v/v) Triton® X-100 in deionized water
- 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 Triton X-100 to a final concentration of 1.67%.
5. Dilute r*Fm*PNGaseF to 0.67 ng/µL in Assay buffer.
6. Combine 15 µL of Substrate Mixture and 15 µL of 0.67 ng/µL r*Fm*PNGaseF. Include a control containing 15 µL of Substrate Mixture and 15 µL of Assay buffer.
7. Incubate 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:

- r*Fm*PNGaseF: 10 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.

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 β-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 α1-3-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.

PRODUCT SPECIFIC NOTICES

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