

Datasheet

Peptide N-Glycosidase A (PNGase A), EC 3.5.1.52

EU219

Peptide-N(4)-(N-acetyl-beta-glucosaminy)asparagine amidase, Glycopeptidase from almonds, almond glycoamidase.

PNGase A from Almond cleaves N-Glycan chains linked to Asparagine from glycopeptides. The enzyme hydrolysis an N(4)-(acetyl-beta-D-glucosaminy) asparagine residue in which the glucosamine residue may be further glycosylated, to yield a (substituted) N-acetyl-beta-D-glucosaminyamine and a peptide containing an aspartate residue. The enzyme cleaves glycopeptides with or without alpha1,3-linked core fucose residues present in insect and plant glycoproteins.

N-glycosidase A requires glycopeptides as substrates. Glycopeptides can be obtained by digesting the glycoprotein with Trypsin as described by Kuester et al. (1997) or pepsin.

Does not act on (GlcNAc)Asn, because it requires the presence of more than two amino-acid residues in the substrate.

Specifications

Catalogue No:	EU219
Packsize:	2 mU/vial OR 10 mU/vial + reaction buffer
Formulation:	Lyophilized powder containing citrate-phosphate buffer, pH 5.0
Reaction buffer:	50 mM citrate-phosphate buffer, pH 5.0, contains 0.02% sodium azide
Remarks:	BSA free. No preservatives added.
Substrate:	N-Glycan chains linked to Asparagine from glycopeptides
Unit definition:	1 U hydrolysis 1.0 μ mol ovalbumin glycopeptide per min at pH 5.0 and 37°C
Specific activity:	> 10 U/mg protein
Contaminants:	each <0.1% of the enzymes given below α - and β -galactosidase, α - and β -mannosidase, β -glucosidase, β -N-acetylhexosaminidase, α -L-fucosidase, sialidase and β -xylosidase
Reconstitution:	Dissolve the enzyme in 40/200 μ L reaction buffer
pH optimum:	4.0-6.0
Recommended reaction temperature:	37°C
Storage:	4°C, do not freeze. Shipping at room temperature.

How to use PNGase A

PNGase A – in contrast to PNGase F - is able to cleave Asn-linked oligosaccharides (N-glycans) also if they contain fucose in α 1,3-linkage to the innermost GlcNAc. It can, however, NOT act on glycoproteins, even if they have been denatured [1][2]. Therefore, glycoproteins must be proteolytically degraded before application of PNGase A.

Option I: PNGase A digestion of tryptic peptides.

This is the safest procedure suitable also for sialylated glycans. Requires protein denaturation (e.g. by reducing SDS-PAGE or even S-alkylation).

Option II: PNGase A digestion of peptidic peptides.

Much simpler but may cause loss of acid labile substituents (sialic acids).

[1] Plummer TH Jr, Tarentino AL (1981) J Biol Chem.256, 10243-6.

[2] Tretter V, Altmann F, März L. (1991) Eur J Biochem. 199, 647-52.

Protocols

Option I: PNGase A digestion of tryptic peptides

- Perform an SDS-PAGE with sample, stain with Coomassie Blue and excise band of interest.
- Destain, (S-alkylate), digest with trypsin and extract peptides according to usual proteomic procedures [1][2]
- Add 0.02 mL of 50 mM citrate-phosphate buffer of pH 5.0
- Incubate at 95° for 5 min to inactivate the protease
- Add 0.05 - 0,1 mU of PNGase A

Oligosaccharides may be purified using carbon solid phase extraction cartridges [3] or passage over reversed phase C18 material [1].

Note 1: If the entire "glycome" of a protein mixture is to be analyzed, electrophoresis can be used just to concentrate and clean the protein plug [4].

Note 2: Use of sequencing grade trypsin is not mandatory.

[1] Kolarich D, Altmann F. (2000) *Analy. Biochem.* 285, 64-75.

[2] Jensen, O. N., Shevchenko, A., and Mann, M. (1997) in *Protein Structure. A*

[3] Packer NH, Lawson MA, Jardine DR, Redmond JW. (1998) *Glycoconjugate J.* 15, 737-47.

[4] Rendić D, Wilson IB, Lubec G, Gutternigg M, Altmann F, Léonard R. (2007) *Electrophoresis* 28, 4484-92.

Option II: PNGase A digestion of peptidic peptides.

- Dissolve glycoprotein in 5 % formic acid (about 1 mL per 2-5 mg of protein)
- Add crystalline porcine pepsin in a ratio of 1 : 50 with regard to substrate protein
- Digest over night at 37°C
- Remove liquid by evaporation under reduced pressure (e.g. Speed-Vac)
- Re-dissolve in a small volume of water (ca. 5 % of original volume) and dry again
- Repeat this step
- Add appropriate amount of 0.1 M citrate buffer of pH 5.0 (about 1 mL per 10 mg of protein)
- Incubate at 95° for 5 min to inactivate pepsin
- Add a minimum of 0.5 mU PNGase A per mg of (original) glycoprotein
- Incubate over night at 37°C

Note: Completeness of digestion should be checked e.g. by thin-layer chromatography using silica plates and a butanol, ethanol, pyridine, acetic acid, water mixture (10 : 100 : 10 : 3 : 30) and any appropriate sugar detection reagent. Also, MALDI-TOF MS may often prove useful.

References

Demonstration of a new amidase acting on glycopeptides

Takahashi, N.; *Biochem. Biophys. Res. Commun.* 76, 1194-1201 (1977)

Some characteristics of a new glycopeptidase acting on aspartylglycosylamine linkages

Takahashi, N.; Nishibe, H.; *J. Biochem.* 84, 1467-1473 (1978)

Complete structure of the carbohydrate moiety of stem bromelain. An application of the almond glycopeptidase for structural studies of glycopeptides

Ishihara, H.; Takahashi, N.; Oguri, S.; Tejima, S.; *J. Biol. Chem.* 254, 10715-10719 (1979)

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The release of carbohydrate moieties from human fibrinogen by almond glycopeptidase without alteration in fibrinogen clottability

Nishibe, H.; Takahashi, N.; *Biochim. Biophys. Acta* 661, 274-279 (1981)

Facile cleavage of complex oligosaccharides from glycopeptides by almond emulsin peptide: N-glycosidase.

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Takahashi, N.; Toda, H.; Nishibe, H.; Yamamoto, K.; *Biochim. Biophys. Acta* 707, 236-242 (1982)

Deglycosylation of asparagine-linked glycans by peptide: N-glycosidase F.

Tarentino, A.L., Gomez, C.M. and Plummer, T.H., Jr., *Biochemistry* 24: 4665-4671 (1985)

Does an animal peptide N:glycanase have the dual role as an enzyme and a carbohydrate binding protein?

Suzuki, T.; Kitajima, K.; Inoue, S.; Inoue, Y.; *Glycoconjugate J.* 11, 469-476 (1994)

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