N-Glycosidase A

Peptide-N-glycosidase A, PNGase A, peptide-N\(^2\)-[N-acetyl-\(\beta\)-glucosaminy] asparagine amidase, EC 3.5.1.52 from sweet almonds

Cat. No. 11 642 995 001
5 mU (100 \(\mu\)l)

Form
Solution in 50 mM citrate/ phosphate buffer, glycerol, 50\% (v/v), pH 5.0.

Specific activity
> 0.5 U/mg protein. One unit is the enzyme activity that hydrolyzes 1 \(\mu\)M ovalbumin glycopeptide Glu-Glu-Lys-Tyr-Asn- (CHO)-Leu-Thr-Ser-Val within 1 min at +37°C at pH 5 at the incubation conditions described below. (CHO consists of hybrid- and high-mannose type oligosaccharides).

Stability
Stable at -15 to -25°C. Repeated freezing and thawing has no effect on the enzyme activity.

Absence of contaminating activities
\(\beta\)-Galactosidase, \(\alpha\)-galactosidase, \(\beta\)-glucosidase, \(\alpha\)- and \(\beta\)-mannosidase, \(\beta\)-N-acetylhexosaminidase, \(\alpha\)-L-fucosidase, \(\beta\)-xylosidase:
Activities measured with the corresponding 4-nitrophenyl glycosides (10 mM each) at +37°C in 100 mM citrate/phosphate buffer, pH 5, are below 0.1%.

Sialidase
Activity measured with 0.1 mM 2'-[(4-methylumbel

Proteases
After incubation of 7 mU N-glycosidase A with 200 \(\mu\)g Universal Protease Substrate, for 17 h at +37°C in 200 \(\mu\)l 50 mM potassium phosphate buffer, pH 7.8, no protease activity is detected, according to the method of Twining (1).

Isolation and properties
N-Glycosidase A is an amidas and cleaves N-glycans between asparagine and the carbohydrate chain, thereby converting asparagine to aspartic acid. The enzyme is an excellent tool to isolate both intact oligosaccharide and peptide moieties for the structural analysis and the functional examination of each moiety. The oligosaccharide is first released as a glycosylamine, which hydrolyzes spontaneously under the acidic conditions of the reaction to the reducing end containing glycan and to ammonia (2). The pH-optimum of the enzyme is very broad between 4.0 and 6.0. Above pH 7.0 the activity decreases rapidly. In contrast, the enzyme retains a partial activity even at pH 2 (2). N-Glycosidase A is purified from sweet almond meal and shows one band corresponding to a molecular weight of 52.5 kDa upon SDS-PAGE (Fig. 1). The enzyme itself is a glycoprotein (4).

Specificity
N-Glycosidase A cleaves all types of asparagine bound N-glycans including high mannannose-, hybrid-, biantennary-, triantennary- and tetraantennary complex types (5), provided that the amino group as well as the carboxyl group are present in peptide linkage. N-Glycosidase A can also cleave a single N-acetylglucosamine residue from the peptide, albeit at a slower reaction rate (6).

In contrast to N-glycosidase F* from Flavobacterium meningosepticum, N-glycosidase A from almonds can degrade N-linked glycans carrying a fucose linked \(\alpha\) (1-3) to Asn-GlcNAc (7). This structural motif is present in plant glycoproteins and is also found in insect glycoproteins.

Deglycosylation
N-Glycosidase A is mainly used for the cleavage of glycopeptides.

1. Incubation conditions for the removal of N-glycans from glycopeptides

For measuring N-glycosidase A activity dilute the enzyme 1:100 in 100 mM citrate/ phosphate buffer, pH 5.0, containing bovine serum albumin (BSA), 0.1\% (v/v). Add 10 \(\mu\)l 100 \(\mu\)M ovalbumin glycopeptide, dissolved in double-distilled water, to 10 \(\mu\)l (5 \(\mu\)l) diluted enzyme and incubate at +37°C for 60 min. Quantify the reaction products by absorption at 220 nm on reversed phase HPLC (5 \(\mu\)M ODS-Hypersil column). The \(K_D\) value for ovabumin glycopeptide is 7 \(\mu\)M.

For preparative digestions incubate 100 nmoles glycopeptide with 0.2 - 0.5 mU N-glycosidase A in 20 - 50 \(\mu\)l buffer without BSA for 24 h at +37°C. To obtain glycopeptides, trypsin sequencing grade, chymotrypsin sequencing grade, or pepsin may be used. These enzymes can be heat-inactivated (5 to 10 min, +100° C) for a direct use in N-glycosidase A digestions.

Figure 1: SDS-PAGE of N-glycosidase A

Lane 1: Molecular weight marker proteins *
Lane 2: 5 mU of N-glycosidase A
2. Incubation conditions for the removal of N-glycans from glycoproteins

Although N-glycosidase A de-glycosylates some glycoproteins in their native form (8), denaturation by using chaotropic salts increases the deglycosylation rate, considerably as shown by Tarentino and Plummer (9). They described the following incubation conditions for the complete deglycosylation of bovine pancreatic ribonuclease B and Fab_{H9262} fragment of immunoglobulin M:

4 μg glycoprotein were treated with 1 mU N-glycosidase A in 10 mM sodium acetate buffer, 0.5 - 0.75 M NaSCN, 0.1 M 2-mercaptoethanol, pH 5.1, for 24 h at +37° C. SDS 0.1% was also used successfully for deglycosylation instead of the chaotropic salt, but under this condition the enzyme was partially inactivated. Protease inhibitors like 10 mM EDTA (4), 4 mM PMSF (4) and 4 mM Pefabloc® SC may be included in the deglycosylation mix and do not inhibit enzyme activity. Some cations such as Mg^{2+}, Zn^{2+}, Co^{3+} and Cu^{2+} increase the enzyme activity by as much as 50% and detergents like 2% Triton X-100 or 2% Tween 80 have also been used for the denaturation of glycoproteins prior to deglycosylation (4).

The extent and rate of deglycosylation of glycoproteins however depend to a high degree on the nature of the glycoprotein. Therefore no general instructions with regard to the incubation conditions can be given.

Changes to Previous Version

- Regulatory Disclaimer updated
- Editorial Changes

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