



# Glycopeptidase A (from Almond)

**Code Number:** 100676

**Size:** 10 mU/vial

**Source:** Almond

**Systematic Name:** *N*-linked - glycopeptide - (*N*- acetyl - β - D - glycosaminy) - L - asparagin amidohydrolase

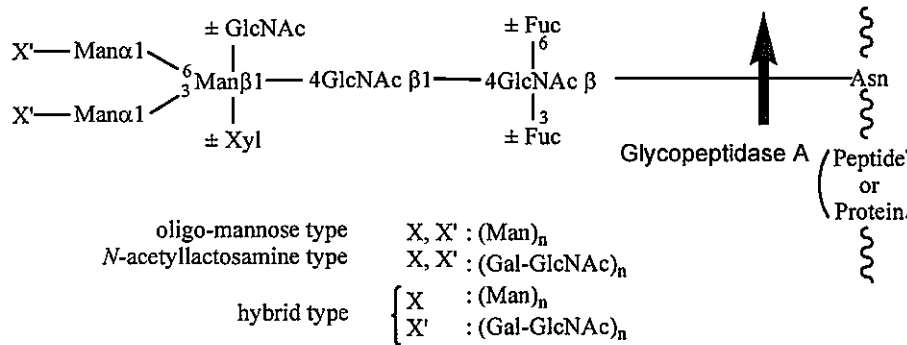
**Alternative Name:** Almond – Glycopeptidase, PNGase A

**EC Number:** 3.5.1.52

**CAS Number:** 83534-39-8

**Description:** Glycopeptidase A, found in the sweet almond, hydrolyzes β - aspartylglycosylamine linkages in glycopeptides to yield equimolar amounts of oligosaccharide, peptide, and ammonia. The enzyme hydrolyzes quantitatively glycopeptides with more than two amino acid residues and various kinds of Asn - linked oligosaccharides [oligo - mannose type, hybrid type and *N* - acetylglucosamine type]. The asparagine residue involved in the linkage converts to an aspartyl residue concurrently with the removal of the oligosaccharide moiety. The enzyme is an excellent tool to isolate intact oligosaccharides for the structural analyses.

**Reaction:**



It acts neither on Asn - oligosaccharide nor on Asn - GlcNAc. The enzyme hydrolyzes intact glycoprotein and directly acts on the cell surface, though the rate of hydrolysis of the glycoproteins is slow.

**Specifications:**

Activity	$\geq 10$ mU/vial
Specific Activity	$\geq 500$ mU/mg protein
Contaminants	β-galactosidase $\leq 1\%$
Appearance	Lyophilized powder containing 10mM Citrate-phosphate buffer, pH5.0
Stabilizer	BSA free
Preservative	None
Reconstitution	Dissolve the enzyme in 200µL of 0.1% BSA
Optimum pH	4.0-6.0 (The activity is lost suddenly over pH7.0)
Recommended Reaction Temperature	37°C
Molecular Weight	66,800 – 79,500
Inhibitors	Cu <sup>2+</sup> , Fe <sup>2+</sup> , Zn <sup>2+</sup> (10mM)
Km value	4mM toward Asn - Asn (Man <sub>2</sub> , Xyl <sub>1</sub> , Fuc <sub>1</sub> , GlcNAc <sub>2</sub> ) - Glu - Ser - Ser.
Stability	Stable at pH 4.0 - 6.0 and at 37°C for 3 days.

AMSBIO | [www.amsbio.com](http://www.amsbio.com) | [info@amsbio.com](mailto:info@amsbio.com)

**UK & Rest of the World**  
 184 Park Drive, Milton Park  
 Abingdon OX14 4SE, UK  
 T: +44 (0) 1235 828 200  
 F: +44 (0) 1235 820 482

**North America**  
 1035 Cambridge Street,  
 Cambridge, MA 02141  
 T: +1 (617) 945-5033 or  
 T: +1 (800) 987-0985  
 F: +1 (617) 945-8218

**Germany**  
 Bockenheimer Landstr. 17/19  
 60325 Frankfurt/Main  
 T: +49 (0) 69 779099  
 F: +49 (0) 69 13376880

**Switzerland**  
 Centro Nord-Sud 2E  
 CH-6934 Bioggio-Lugano  
 T: +41(0) 91 604 55 22  
 F: +41(0) 91 605 17 85

**Unit Definition:** One enzyme unit is defined as the amount of the enzyme required to hydrolyze 1µmole of ovalbumin glycopeptide, Glu - Glu (or Gln) - Lys - Tyr - Asn (Man<sub>5</sub>, GlcNAc<sub>3</sub>) - Leu - Thr - Ser - Val, at pH 5.0 and at 37°C per minute.

**Assay for Enzyme Activity:**

<b>Method:</b>		
[Reaction mixture]		
Substrate and Buffer solution:	Samples containing glycopeptides (0.1µmole) in 0.1M Citrate phosphate buffer, pH 5.0	20µL
Enzyme solution:	Suitably diluted enzyme (0.1-1mU) with 0.1% BSA	5µL
Total volume		25µL
[Procedure]		
Reaction: The reaction mixture is incubated for 5–16 hours at 37°C.		

**Application:**

- Structural analysis of *N*-linked glycoproteins
- Functional studies of *N*-linked glycoproteins
- Mechanism of biosynthesis pathways

**Storage:** Store at -20°C until opened. Following reconstitution, aliquot and freeze (-20°C).

**References:**

**A) Purification and characterization of the enzyme**

- 1) Takahashi, N.: *Biochem. Biophys. Res. Commun.*, **76**, 1194 (1977)
- 2) Takahashi, N. and Nishibe, H.: *Biochim. Biophys. Acta.*, **657**, 457 (1981)
- 3) Sugiyama, K., Ishihara, H., Tejima, S. and Takahashi, N.: *Biochem. Biophys. Res. Commun.*, **112**, 155 (1983)
- 4) Plummer, T. H. Jr. and Tarentino, A. L.: *J. Biol. Chem.*, **256**, 10243 (1981)
- 5) Taga, E. M., Waheed, A. and Van Etten, R. L.: *Biochemistry*, **23**, 815 (1984)
- 6) Risley, J. M. and Van Etten, R. L.: *J. Biol. Chem.*, **260**, 15488 (1985)

**B) Structural studies of the oligosaccharides**

- 7) Takahashi, N., Hotta, T., Ishihara, H., Mori, M., Tejima, S., Bligny, R., Akazawa, S., Endo, S. and Arata, Y.: *Biochemistry*, **25**, 388 (1986)
- 8) Nomoto, H., Takahashi, N., Nagaki, Y., Endo, S., Arata, Y. and Hayashi, K.: *Eur. J. Biochem.*, **157**, 233 (1986)
- 9) Takahashi, N., Ishii, I., Ishihara, H., Mori, M., Tejima, S., Jefferis, R., Endo, S. and Arata, Y.: *Biochemistry*, **26**, 1137 (1987)
- 10) Tsuda, E., Goto, M., Murakami, A., Akai, K., Ueda, M., Kawanishi, G., Takahashi, N., Sasaki, R., Chiba, H., Ishihara, H., Mori, M., Tejima, S., Endo, S. and Arata, Y.: *Biochemistry*, **27**, 5646 (1988)
- 11) Tomiya, N., Yamaguchi, T., Awaya, J., Kurono, M., Endo, S., Arata, Y., Takahashi, N., Ishihara, H., and Tejima, S.: *Biochemistry*, **27**, 7146 (1988)
- 12) Tomiya N., Awaya, J., Kurono, M., Endo, S., Arata, Y. and Takahashi, N.: *Anal. Biochem.*, **171**, 73 (1988)
- 13) Swiedler, S. J., Hart, G. W., Tarentino, A. L., Plummer, T. H. Jr. and Freed, J. H.: *J. Biol. Chem.*, **258**, 11515 (1983)
- 14) Torres, C. R. and Hart, G. W.: *J. Biol. Chem.*, **259**, 3308 (1984)
- 15) Swiedler, S. J., Freed, J. H., Tarentino, A. L., Plummer, T. H. Jr. and Hart, G. W.: *J. Biol. Chem.*, **260**, 4046 (1985)

**C) Removal of sugars from intact glycoproteins**

- 16) Nishibe, H. and Takahashi, N.: *Biochim. Biophys. Acta.*, **661**, 274 (1981)
- 17) Takata, K., Yamamoto, K - Y., Ishii, I. and Takahashi, N.: *Cell Differentiation*, **14**, 25 (1984)
- 18) Tarentino, A. L. and Plummer, T. H. Jr.: *J. Biol. Chem.*, **257**, 10776 (1982)

**Note:** For *in vitro* research use only, not for diagnostic or therapeutic use. This product is not medical device.