

## Glycan Recognizing Proteins

### Galectins

Galectins are a family of animal carbohydrate binding proteins; the name is from their description as  $\beta$ -galactoside-specific **lectins**. They have been strongly implicated in inflammation and cancer and may be useful as targets for the development of new anti-inflammatory and anti-cancer therapies.

Galectins occur at high concentration in a limited range of cell types, different for each galectin. Galectins bind to sugar molecules on the surface of cells. All galectins bind lactose and other  $\beta$ -galactosides, but they differ in their affinity for more complex saccharides.<sup>1</sup> The galectins are defined by their structural similarities in their carbohydrate recognition domains (CRD) and by their affinity for  $\beta$ -galactosides; fourteen human members have been reported so far.<sup>2</sup> The galectins have been classified into three classes: prototype, chimera, and tandem-repeat galectins. The prototype galectins-1, -2, -5, -7, -10, -11, -13 and -14, all contain one CRD and are either monomers or noncovalent homodimers. The only chimera galectin currently identified (galectin-3) contains one CRD connected to a non-lectin domain. The tandem-repeat galectins-4, -6, -8, -9, and -12 consist of two CRDs joined by a linker peptide.

Extracellular galectins crosslink cell-surface and extracellular glycoproteins and may thereby modulate cell adhesion and induce intracellular signals. Galectins may also bind intracellular non-carbohydrate ligands and have intracellular regulatory roles in processes such as RNA splicing, apoptosis, and, suggested most recently, the cell cycle.<sup>1</sup>

#### Galectin-1

Galectin-1 has been implicated in metastasis and aggregation of cancer cells based on its association with the glycoprotein 90K.<sup>4,5</sup> It has been shown to induce apoptosis of activated T cells,<sup>6</sup> T-leukemia cell lines,<sup>7</sup> breast,<sup>8</sup> colon,<sup>9</sup> and prostate<sup>10</sup> cancer cells. Other activities of galectin-1 include cell differentiation and inhibition of CD45 protein phosphatase activity. Galectin-1 binds CD45, CD3, and CD4 in addition to  $\beta$ -galactoside. Galectin-1 bound in the extracellular matrix can induce cell death of adherent T cells at a ten-fold lower concentration than soluble galectin-1.<sup>11</sup> Galectin-1 may play a significant role in cancer through apoptosis, cell adhesion and migration, regulation of the cell cycle, and tumor evasion of immune responses.<sup>12,13</sup>

#### Galectin-3

Galectin-3, also called Mac-2, L29, CBP35 and  $\epsilon$ BP, is a chimera galectin that is expressed in tumor cells, macrophages, activated T cells, epithelial cells, and fibroblasts. It binds a variety of matrix glycoproteins including laminin and fibronectin. Intracellularly, galectin-3 acts to prevent apoptosis. Depending on the cell type, galectin-3 can be localized in the extracellular matrix, the cell surface, in the cytoplasm, or in the nucleus. Galectin-3 has been shown to exhibit proinflammatory activities *in vitro* and *in vivo*;<sup>14</sup> it induces pro-inflammatory responses and inhibits Th2

type cytokine production.<sup>3</sup> High levels of circulating galectin-3 have been shown to correlate with the malignancy potential of several types of cancer.<sup>15-17</sup> Galectin-3 is known to play a role in tumor growth, metastasis, and cell-to-cell adhesion. It also serves as a preferred substrate for matrix metalloproteinase-9 (MMP-9).<sup>18</sup> Human and mouse galectin-3 share approximately 80% homology in their amino acid sequence.<sup>19</sup>

#### Galectin-3C

Galectin-3C is a truncated form of galectin-3 that contains the carboxy-terminus carbohydrate-binding domain. Recombinant galectin-3C competes with endogenous galectin-3 for carbohydrate binding sites and acts as a negative inhibitor of galectin-3<sup>20</sup> in promoting cell adhesion<sup>21</sup> and cell signaling. Galectin-3C has been found to be effective in reducing metastases and tumor volumes and weights in primary tumors in an orthotopic nude mouse model of human breast cancer.<sup>22</sup>

#### Galectin-8

Galectin-8, also known as prostate carcinoma tumor antigen 1 (PCTA1) in human, is a tandem repeat-type galectin. High levels of circulating galectin-8 have been shown to correlate with lung carcinomas, certain forms of prostate carcinomas, as well as other tumor cells.<sup>23</sup> It binds to a subset of cell surface integrins to modulate ECM-integrin interactions. It acts as a physiological modulator of cell adhesion and cellular growth, and may be involved in neoplastic transformation.<sup>24-26</sup> Human and mouse galectin-8 share approximately 80% homology in their amino acid sequence.<sup>19</sup>

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Name	Recombinant Host	Assay	Form	Cat. No.
Galectin-1 human	<i>Escherichia coli</i>	$\geq 95\%$ , SDS-PAGE	Lyophilized from a 5 mg/mL solution in phosphate buffered saline containing 8 mM DTT and 150 mM lactose.	<b>NEW</b> G7420-250UG
Galectin-3 human	<i>Escherichia coli</i>	-	The product is lyophilized with 200 $\mu$ g of lactose as stabilizer per 100 $\mu$ g vial of galectin-3.	G5170-100UG
Galectin-3C human	<i>Escherichia coli</i>	-	The product is lyophilized with 200 $\mu$ g of lactose as stabilizer per 100 $\mu$ g vial of galectin-3C.	G5295-100UG
Galectin-8 from rat	<i>Escherichia coli</i>	$\geq 90\%$ , SDS-PAGE	Solution containing 20 mM Tris, pH 7.4, 1 mM DTT, 1 mM EDTA, and 30% glycerol.	G3670-100UG

# Lectins

## Lectins

Lectins are proteins or glycoproteins from non-immune origins that agglutinate cells and/or precipitate complex carbohydrates. Lectins are isolated from a wide variety of natural sources, both plant and animal. Recombinant human and rat galectins are expressed in *Escherichia coli*. The agglutination activity of these highly specific carbohydrate-binding molecules is usually inhibited by a simple monosaccharide, but for some lectins di-, tri-, and even polysaccharides are required. Sigma offers a wide range of lectins suitable for the following applications:

- Carbohydrate studies
- Fractionation of cells and other particles
- Lymphocyte subpopulation studies
- Mitogenic stimulation
- Blood group typing
- Histochemical studies

Lectin Source	Acronym	Mol. Wt. (kDa)	Subunits	Blood Group Specificity	Carbohydrate Specificity	Mitogenicity	Protein Families <sup>a</sup>	Related Domains <sup>b</sup>
<b>Agaricus bisporus</b> (Mushroom)	ABA	58.5	—	—	$\beta$ -Gal(1 $\rightarrow$ 3)GalNAc		Fungal fruit body lectin	Fungal fruit body lectin
<b>Arachis hypogaea</b> (Peanut)	PNA	120	4	T	$\beta$ -Gal(1 $\rightarrow$ 3)GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Artocarpus integrifolia</b> (Jacalin)		42	4	T	$\alpha$ -Gal-OME	Mitogenic	Jacalin-like lectin domain	Jacalin-like lectin domain
<b>Bandeiraea simplicifolia</b> (Griffonia simplicifolia)	BS-I	114	4	A, B	$\alpha$ -Gal, $\alpha$ -GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Isolectin A<sub>4</sub></b>	BS-I-A4	114	4	A	$\alpha$ -GalNAc		Not reported	Not reported
<b>Isolectin B<sub>4</sub></b>	BS-I-B4	114	4	B	$\alpha$ -Gal		Not reported	Not reported
<b>Caragana arborescens</b> (Siberian pea tree)		60;120 <sup>c</sup>	2;4	—	GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Cicer arietinum</b> (Chick pea)		44	2	—	Fetuin		Not reported	Not reported
<b>Codium fragile</b> (Green marine algae)		60	4	—	GalNAc		Not reported	Not reported
<b>Concanavalin A from Canavalia ensiformis</b> (Jack bean) (Con A)		102	4	—	$\alpha$ -Man, $\alpha$ -Glc	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Succinyl-Concanavalin A</b>		51	2	—	$\alpha$ -Man, $\alpha$ -Glc	Mitogenic <sup>d</sup>	Not reported	Not reported
<b>Datura stramonium</b> (Jimson weed; Thorn apple)	DSL	86	2( $\alpha$ & $\beta$ ) <sup>e</sup>	—	(GlcNAc) <sub>2</sub>		Not reported	Not reported
<b>Dolichos biflorus</b> (Horse gram)	DBA	140	4	A <sub>1</sub>	$\alpha$ -GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Erythrina cristagalli</b> (Coral tree)	ECA	56.8	2( $\alpha$ & $\beta$ ) <sup>e</sup>	—	$\beta$ -Gal(1 $\rightarrow$ 4)GlcNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Galanthus nivalis</b> (Snowdrop)	GNL	52	4	Rabbit <sup>f</sup>	non-reduc. D-Man		Curculin-like (mannose-binding) lectin	D-mannose binding lectin

Lectin Source	Acronym	Mol. Wt. (kDa)	Subunits	Blood Group Specificity	Carbohydrate Specificity	Mitogenicity	Protein Families <sup>a</sup>	Related Domains <sup>b</sup>
<b>Glycine max</b> (Soybean)	SBA	110	4	—	GalNAc	Mitogenic <sup>d</sup>	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Helix aspersa</b> (Garden snail)	HAA	79	—	A	GalNAc		Not reported	Not reported
<b>Helix pomatia</b> (Edible snail)	HPA	79	6	A	GalNAc		Not reported	Not reported
<b>Human Galectin-1</b>	Gal-1	14	2	—	$\beta$ -Gal		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Galectin, galactose-binding lectin	Galactoside binding lectin domain
<b>Human Galectin-3</b>	Gal-3	26	—	—	$\beta$ -Gal		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Galectin, galactose-binding lectin	Galactoside binding lectin domain
<b>Human Galectin-3C</b>		16	—	—	$\beta$ -Gal		Not reported	Not reported
<b>Lens culinaris</b> (Lentil)	LcH	49	2	—	$\alpha$ -Man		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Limulus polyphemus</b> (Horseshoe crab)		400	18	—	NeuNAc		Not reported	Lectin-type C domain
<b>Lycopersicon esculentum</b> (Tomato)	LEA	71	—	—	(GlcNAc) <sub>3</sub>	Mitogenic <sup>h</sup>	Not reported	Galactose binding lectin domain
<b>Maackia amurensis</b>	MAA	130	2( $\alpha$ & $\beta$ )	O	$\alpha$ -Neu NAC (2 $\rightarrow$ 3)Gal	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Phaseolus vulgaris Erythroagglutinin</b> (Red kidney bean)	PHA-E	128	4	—	Oligosaccharide	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Phaseolus vulgaris Leucoagglutinin</b> (Red kidney bean)	PHA-L	126	4	—	Oligosaccharide	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Phaseolus vulgaris Phytohemagglutinin</b> (red kidney bean)	PHA-P						Not reported	Not reported
<b>Phaseolus vulgaris Mucoprotein</b> (red kidney bean)	PHA-M						Not reported	Not reported
<b>Phytolacca americana</b> (Pokeweed)	PWM	32 <sup>i</sup>	—	—	(GlcNAc) <sub>3</sub>	Mitogenic	Chitin recognition protein	Chitin-binding, type 1
<b>Pisum sativum</b> (Garden pea)	PSA	49	4( $\alpha$ & $\beta$ ) <sup>e</sup>	—	$\alpha$ -Man	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain
<b>Pseudomonas aeruginosa</b>	PA-I	13-13.7	—	—	Gal	Mitogenic <sup>g</sup>	Galactose-binding like	PA-IL-like protein
<b>Psophocarpus tetragonolobus</b> (Winged bean)		35	1	—	GalNAc, Gal		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, $\alpha$ Legume lectin, $\beta$ domain	Legume lectin domain

# Lectins

Lectin Source	Acronym	Mol. Wt. (kDa)	Subunits	Blood Group Specificity	Carbohydrate Specificity	Mitogenicity	Protein Families <sup>a</sup>	Related Domains <sup>b</sup>
<b>Rat Galectin-8</b>	Gal-8	34	—	—	β-Gal	—	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Galectin, galactose-binding lectin	Galactoside binding lectin domain
<b>Ricinus communis Agglutinin</b> (Castor bean)	RCA <sub>120</sub>	120	4	—	β-Gal	—	Ricin B lectin Ricin B-related lectin Ribosome-inactivating protein	Ricin-type β-trefoil lectin domain Ribosome inactivating protein (RIP)
<b>Ricinus communis Ricin, A chain</b> (Castor bean)	—	—	—	—	—	—	Ricin B lectin Ricin B-related lectin Ribosome-inactivating protein	Ricin-type β-trefoil lectin domain Ribosome inactivating protein (RIP)
<b>Sambucus nigra</b> (Elder)	SNA	140	4(α&β) <sup>e</sup>	-	α-NeuNAc(2→6) Gal/GalNAc	Mitogenic <sup>g</sup>	Ricin B lectin Ricin B-related lectin	Ricin-type β-trefoil lectin domain
<b>Solanum tuberosum</b> (Potato)	STA	50; 100 <sup>c</sup>	1;2	-	(GlcNAc) <sub>3</sub>	—	Jacalin-related lectin	Jacalin-like lectin domain
<b>Tetragonolobus purpureas</b> (Lotus tetragonolobus, winged or asparagus pea)	—	120(A), 58 (B), 117(C)	4;2;4	H	α-L-Fuc	—	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain
<b>Triticum vulgare</b> (Wheat germ)	WGA	36	2	—	(GlcNAc) <sub>2</sub> , NeuNAc	—	Chitin recognition protein	Chitin-binding, type 1
<b>Ulex europaeus</b> (Gorse)	UEA I	68	—	H	α-L-Fuc	—	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain
<b>Vicia villosa</b> (Hairy vetch)	VVA	139	4 <sup>e</sup>	A <sub>1</sub> +T <sub>n</sub>	GalNAc	—	Not reported	Not reported
<b>Vicia villosa Isolectin B4</b>	—	143	4	T <sub>n</sub>	GalNAc	—	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	—
<b>Viscum album</b> (Mistletoe)	—	115 <sup>i</sup>	4(αβ) <sup>e</sup>	—	β-Gal	—	Ricin B lectin Ricin B-related lectin	Ricin-type β-trefoil lectin domain
<b>Wisteria floribunda</b>	WFA	68	2	—	GalNAc	—	Not reported	Not reported
<b>Wisteria floribunda, Reduced</b>	—	34	1	—	GalNAc	—	Not reported	Not reported

## Notes:

- Swiss Institute of Bioinformatics Swiss-Prot/European Bioinformatics Institute InterPro protein sequence database
- Wellcome Trust Sanger Institute Pfam protein sequence database
- Concentration-dependent mol. wt. change
- Non-agglutinating and mitogenic
- Subunits are of different molecular weights
- Agglutinates rabbit, but not human, erythrocytes
- Mitogenic for neuraminidase-treated lymphocytes
- Inhibits mitogenic activity of PHA
- Data given for PWM Pa2
- Data given for VAA(I)

*Agaricus bisporus* (Mushroom)

Name	Form	Cat. No.
Lectin from <i>Agaricus bisporus</i> (mushroom)	lyophilized powder	L5640-1MG L5640-2MG

*Arachis hypogaea* (Peanut)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Arachis hypogaea</i> (peanut)	-	-	lyophilized powder	L0881-5MG L0881-10MG L0881-25MG
Lectin from <i>Arachis hypogaea</i> (peanut)	biotin conjugate	biotin ~ 3 mol per 1 mol	lyophilized powder	L6135-1MG L6135-5MG
Lectin from <i>Arachis hypogaea</i> (peanut)	FITC conjugate	FITC 4 - 8 mol per 1 mol	lyophilized powder	L7381-1MG L7381-2MG L7381-5MG
Lectin from <i>Arachis hypogaea</i> (peanut)	peroxidase conjugate	-	lyophilized powder	L7759-250UG L7759-1MG
Lectin from <i>Arachis hypogaea</i> (peanut)	TRITC conjugate	TRITC 1.5 - 3 mol per 1 mol	lyophilized powder	L3766-2MG
Lectin from <i>Arachis hypogaea</i> (peanut)	agarose conjugate	2 - 4 mg/mL	saline suspension	L2507-1ML L2507-2ML

*Artocarpus integrifolia* (Jacalin)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Artocarpus integrifolia</i>	-	-	lyophilized powder	L3515-10MG
Lectin from <i>Artocarpus integrifolia</i>	agarose conjugate	~ 5 mg/mL	saline suspension	L5147-2ML L5147-5ML

*Bandeiraea simplicifolia* (*Griffonia simplicifolia*)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> )	-	-	lyophilized powder	L2380-1MG L2380-5MG
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> )	biotin conjugate	biotin ~ 5 mol per 1 mol	lyophilized powder	L3759-1MG
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> )	FITC conjugate	FITC 1 - 3 mol per 1 mol	lyophilized powder	L9381-2MG
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> )	TRITC conjugate	TRITC 1.5 - 3.5 mol per 1 mol	lyophilized powder	L5264-2MG
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> )	-	-	lyophilized powder	L3019-1MG
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> ), Isolectin B <sub>4</sub>	biotin conjugate	biotin 2 - 4 mol per 1 mol	lyophilized powder	L2140-.2MG L2140-1MG
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> ), Isolectin B <sub>4</sub>	FITC conjugate	FITC ≥ 1 mol per 1 mol	lyophilized powder	L2895-.2MG L2895-1MG
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> ), Isolectin B <sub>4</sub>	peroxidase conjugate	-	lyophilized powder	L5391-.2MG L5391-.5MG
Lectin from <i>Bandeiraea simplicifolia</i> ( <i>Griffonia simplicifolia</i> ), Isolectin A <sub>4</sub>	FITC conjugate	FITC 2 - 4 mol per 1 mol	lyophilized powder	L0890-.2MG L0890-1MG

*Caragana arborescens* (Siberian pea tree)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Caragana arborescens</i> (Siberian pea tree)	biotin conjugate	biotin 3 - 6 mol per 1 mol	lyophilized powder	L9637-1MG

*Cicer arietinum* (chick pea)

Name	Form	Cat. No.
Lectin from <i>Cicer arietinum</i> (chick pea)	lyophilized powder	L3141-5MG

# Lectins

## *Codium fragile* (Green marine algae)

Name	Form	Cat. No.
Lectin from <i>Codium fragile</i> (green marine algae)	lyophilized powder	L2638-5MG L2638-1MG L2638-5MG

## Concanavalin A (Con A)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type VI	-	-	lyophilized powder	L7647-25MG L7647-100MG L7647-250MG L7647-1G
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type V	-	-	lyophilized powder	C7275-25MG C7275-100MG C7275-250MG C7275-1G
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type IV	-	-	lyophilized powder	C2010-25MG C2010-100MG C2010-250MG C2010-1G
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type IV-S cell culture tested, aseptically processed	-	-	lyophilized powder	C5275-5MG
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type IV-S cell culture tested, $\gamma$ -irradiated	-	-	lyophilized powder	C0412-5MG
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type IV	biotin conjugate	biotin 4 - 8 mol per 1 mol	lyophilized powder	C2272-2MG C2272-10MG
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type IV	ferritin conjugate	ferritin ~ 1 mol per 1 mol	buffered aqueous glycerol solution	C7898-5MG C7898-10MG
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type IV	FITC conjugate	FITC 3 - 6 mol per 1 mol	lyophilized powder	C7642-2MG C7642-10MG
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean)	peroxidase conjugate	-	lyophilized powder	L6397-1MG L6397-5MG
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean)	Sepharose® conjugate	10 - 15 mg/mL	buffered aqueous suspension	C9017-25ML C9017-100ML
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean), Type V-A	agarose conjugate	~ 10 mg/mL	buffered aqueous suspension	C6904-5ML C6904-10ML
Succinyl-Concanavalin A	-	-	lyophilized powder	L3885-25MG

## *Datura stramonium* (Jimson weed; thorn apple)

Name	Form	Cat. No.
Lectin from <i>Datura stramonium</i> (jimson weed, thorn apple)	lyophilized powder	L2766-1MG L2766-5MG

## *Dolichos biflorus* (Horse gram)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Dolichos biflorus</i> (horse gram)	-	-	lyophilized powder	L2785-1MG L2785-5MG
Lectin from <i>Dolichos biflorus</i> (horse gram)	agarose conjugate	protein (Lowry) 2 - 4 mg/mL	buffered aqueous suspension	L9894-1ML
Lectin from <i>Dolichos biflorus</i> (horse gram)	biotin conjugate	biotin $\geq$ 3 mol per 1 mol	lyophilized powder	L6533-1MG L6533-5MG
Lectin from <i>Dolichos biflorus</i> (horse gram)	FITC conjugate	FITC 3 - 8 mol per 1 mol	lyophilized powder	L9142-1MG
Lectin from <i>Dolichos biflorus</i> (horse gram)	peroxidase conjugate	-	lyophilized powder	L1287-250UG
Lectin from <i>Dolichos biflorus</i> (horse gram)	TRITC conjugate	TRITC 1 - 3 mol per 1 mol	lyophilized powder	L9658-2MG

*Erythrina cristagalli* (Coral tree)

Name	Form	Cat. No.
Lectin from <i>Erythrina cristagalli</i> (coral tree)	lyophilized powder	L5390-5MG L5390-10MG

*Galanthus nivalis* (Snowdrop)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Galanthus nivalis</i> (snowdrop)	-	-	lyophilized powder	L8275-5MG
Lectin from <i>Galanthus nivalis</i> (snowdrop)	agarose conjugate	protein 2 - 4 mg/mL	saline suspension	L8775-1ML L8775-2ML

*Glycine max* (Soybean)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Glycine max</i> (soybean)	-	-	lyophilized powder	L1395-5MG L1395-25MG
Lectin from <i>Glycine max</i> (soybean)	peroxidase conjugate	-	lyophilized powder	L2650-1MG
Lectin from <i>Glycine max</i> (soybean)	TRITC conjugate	TRITC ~ 1 mol per 1 mol	lyophilized powder	L4511-2MG

*Helix aspersa* (Garden snail)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Helix aspersa</i> (garden snail)	-	-	lyophilized powder	L6635-1MG
Lectin from <i>Helix aspersa</i> (garden snail)	biotin conjugate	biotin 1.5 - 5.4 mol per 1 mol	lyophilized powder	L8764-1MG

*Helix pomatia*

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Helix pomatia</i>	-	-	lyophilized powder	L3382-1MG L3382-5MG
Lectin from <i>Helix pomatia</i>	-	-	lyophilized powder	L2890-1VL
Lectin from <i>Helix pomatia</i>	biotin conjugate	biotin 2 - 4 mol per 1 mol	lyophilized powder	L6512-1MG
Lectin from <i>Helix pomatia</i>	FITC conjugate	FITC 2 - 5 mol per 1 mol	buffered aqueous solution	L1034-1ML
Lectin from <i>Helix pomatia</i>	peroxidase conjugate	-	lyophilized powder	L6387-5MG L6387-1MG
Lectin from <i>Helix pomatia</i>	TRITC conjugate	TRITC ~ 1 mol per 1 mol	lyophilized powder	L1261-1MG
Lectin from <i>Helix pomatia</i>	agarose conjugate	lectin 1 - 3 mg/mL	saline suspension	L8639-1ML L8639-5ML

*Lens culinaris* (Lentil)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Lens culinaris</i> (lentil)	-	-	lyophilized powder	L9267-5MG L9267-25MG L9267-100MG
Lectin from <i>Lens culinaris</i> (lentil)	biotin conjugate	biotin 2 - 5 mol per 1 mol	lyophilized powder	L4143-1MG
Lectin from <i>Lens culinaris</i> (lentil)	FITC conjugate	FITC ~ 2 mol per 1 mol	lyophilized powder	L9262-1MG L9262-2MG L9262-5MG
Lectin from <i>Lens culinaris</i> (lentil)	agarose conjugate	2 - 4 mg/mL	saline suspension	L4018-1ML L4018-5ML

*Limulus polyphemus* (Horseshoe crab)

Name	Form	Cat. No.
Lectin from <i>Limulus polyphemus</i> (horseshoe crab)	lyophilized powder	L2263-1MG

# Lectins

## *Lycopersicon esculentum* (Tomato)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Lycopersicon esculentum</i> (tomato)	-	-	lyophilized powder	L2886-1MG L2886-5MG
Lectin from <i>Lycopersicon esculentum</i> (tomato)	biotin conjugate	biotin 3 - 5 mol per 1 mol	lyophilized powder	L0651-1MG L0651-2MG
Lectin from <i>Lycopersicon esculentum</i> (tomato)	FITC conjugate	FITC 2 - 6 mol per 1 mol	buffered aqueous solution	L0401-1MG

## *Maackia amurensis*

Name	Form	Cat. No.
Lectin from <i>Maackia amurensis</i>	lyophilized powder	L8025-5MG

## *Phaseolus vulgaris* (Red kidney bean)

Name	Description	Form	Cat. No.
Lectin from <i>Phaseolus vulgaris</i> (red kidney bean)	Erythroagglutinin PHA-E	salt-free, lyophilized powder	L8629-5MG
Lectin from <i>Phaseolus vulgaris</i> (red kidney bean)	Leucoagglutinin PHA-L	lyophilized powder	L2769-2MG L2769-5MG L2769-10MG
Lectin from <i>Phaseolus vulgaris</i> (red kidney bean), cell culture tested	Leucoagglutinin PHA-L	lyophilized powder	L4144-5MG
Lectin from <i>Phaseolus vulgaris</i> (red kidney bean)	Phytohemagglutinin PHA-P	lyophilized powder	L8754-5MG L8754-25MG L8754-50MG
Lectin from <i>Phaseolus vulgaris</i> (red kidney bean), purified by affinity chromatography	Phytohemagglutinin PHA-P	lyophilized powder	L9017-1MG L9017-5MG L9017-10MG
Lectin from <i>Phaseolus vulgaris</i> (red kidney bean), cell culture tested	Phytohemagglutinin PHA-P	lyophilized powder (contains buffer salts and NaCl)	L1668-5MG
Lectin from <i>Phaseolus vulgaris</i> (red kidney bean)	Phytohemagglutinin PHA-M	lyophilized powder	L2646-10MG L2646-25MG L2646-100MG
Lectin from <i>Phaseolus vulgaris</i> (red kidney bean), cell culture tested	Phytohemagglutinin PHA-M	lyophilized powder	L8902-5MG L8902-25MG L8902-100MG
Lectin from <i>Phaseolus vulgaris</i>	-	powder	61764-1MG 61764-5MG

## *Phytolacca americana* (Pokeweed)

Name	Form	Cat. No.
Lectin from <i>Phytolacca americana</i> (pokeweed)	lyophilized powder	L9379-5MG L9379-10MG L9379-40MG

## *Pisum sativum* (Pea)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Pisum sativum</i> (pea)	-	-	lyophilized powder	L5380-5MG
Lectin from <i>Pisum sativum</i> (pea)	FITC conjugate	FITC 2 - 4 mol per 1 mol	lyophilized powder	L0770-2MG

## *Pseudomonas aeruginosa*

Name	Form	Cat. No.
Lectin from <i>Pseudomonas aeruginosa</i>	lyophilized powder	L9895-1MG L9895-2MG

*Psophocarpus tetragonolobus* (Winged bean)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Psophocarpus tetragonolobus</i> (winged bean)	biotin conjugate	biotin ~ 3 mol per 1 mol	lyophilized powder	L3014-1MG
Lectin from <i>Psophocarpus tetragonolobus</i> (winged bean)	FITC conjugate	FITC ~ 3 mol per 1 mol	lyophilized powder	L3264-5MG
Lectin from <i>Psophocarpus tetragonolobus</i> (winged bean)	TRITC conjugate	-	lyophilized powder	L3389-5MG

*Ricinus communis* (Castor bean)

Name	Description	Form	Cat. No.
Lectin from <i>Ricinus communis</i> (castor bean) Agglutinin - RCA <sub>120</sub>	-	buffered aqueous solution	L7886-5MG L7886-10MG
Ricin A chain from <i>Ricinus communis</i> (castor bean)	-	buffered aqueous glycerol solution	L9514-1MG L9514-5MG
Ricin A chain from <i>Ricinus communis</i> (castor bean)	deglycosylated	buffered aqueous glycerol solution	L4022-1MG

*Sambucus nigra* (Elder)

Name	Form	Cat. No.
Lectin from <i>Sambucus nigra</i> (elder)	lyophilized powder	L6890-1MG L6890-2MG

*Solanum tuberosum* (Potato)

Name	Form	Cat. No.
Lectin from <i>Solanum tuberosum</i> (potato)	lyophilized powder	L4266-5MG

*Tetragonolobus purpureas* (Lotus tetragonolobus, winged or asparagus pea)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Tetragonolobus purpureas</i> (Lotus tetragonolobus, winged or asparagus pea)	-	-	lyophilized powder	L9254-5MG
Lectin from <i>Tetragonolobus purpureas</i> (Lotus tetragonolobus, winged or asparagus pea)	biotin conjugate	biotin ~ 6 mol per 1 mol	lyophilized powder	L3134-1MG L3134-5MG

*Triticum vulgare* (Wheat)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Triticum vulgare</i> (wheat)	-	-	lyophilized powder	L9640-10MG L9640-25MG L9640-100MG
Lectin from <i>Triticum vulgare</i> (wheat) cell culture tested	-	-	lyophilized powder	L0636-5MG
Lectin from <i>Triticum vulgare</i> (wheat)	biotin conjugate	biotin 2 - 4 mol per 1 mol	lyophilized powder	L5142-1MG L5142-5MG
Lectin from <i>Triticum vulgare</i> (wheat)	Evans blue conjugate	Evans blue ~ 2 mol per 1 mol	lyophilized powder	L9884-1MG
Lectin from <i>Triticum vulgare</i> (wheat)	FITC conjugate	FITC ~ 2 mol per 1 mol	lyophilized powder	L4895-2MG L4895-5MG L4895-10MG
Lectin from <i>Triticum vulgare</i> (wheat)	peroxidase conjugate	-	lyophilized powder	L3892-1MG L3892-2MG L3892-5MG
Lectin from <i>Triticum vulgare</i> (wheat)	agarose conjugate	~ 6 mg/mL	saline suspension	L1394-1ML L1394-5ML L1394-10ML
Lectin from <i>Triticum vulgare</i> (wheat)	agarose conjugate	5 - 10 mg/mL	saline suspension	L1882-1ML L1882-5ML
Lectin from <i>Triticum vulgare</i> (wheat)	TRITC conjugate	TRITC ~ 1 mol per 1 mol	lyophilized powder	L5266-2MG

## Lectins

### *Ulex europaeus* (Gorse, furze)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Ulex europaeus</i> (gorse, furze)	-	-	lyophilized powder	L5505-2MG L5505-5MG
Lectin from <i>Ulex europaeus</i> (gorse, furze)	biotin conjugate	biotin ~ 3 mol per 1 mol	lyophilized powder	L8262-.5MG L8262-2MG
Lectin from <i>Ulex europaeus</i> (gorse, furze)	FITC conjugate	FITC 2 - 5 mol per 1 mol	lyophilized powder	L9006-1MG L9006-2MG
Lectin from <i>Ulex europaeus</i> (gorse, furze)	peroxidase conjugate	-	lyophilized powder	L8146-.5MG L8146-1MG
Lectin from <i>Ulex europaeus</i> (gorse, furze)	TRITC conjugate	TRITC 4 - 8 mol per 1 mol	lyophilized powder	L4889-.5MG

### *Vicia villosa* (Hairy vetch)

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Vicia villosa</i> (hairy vetch)	agarose conjugate	2 - 4 mg/mL	aqueous glycerol suspension	L9388-5MG
Lectin from <i>Vicia villosa</i> (hairy vetch), Isolectin B <sub>4</sub>	-	-	lyophilized powder	L7513-1MG L7513-5MG


### *Viscum album* (European mistletoe)


Name	Form	Cat. No.
Lectin from <i>Viscum album</i> (European mistletoe)	lyophilized powder	L2662-1MG

### *Wisteria floribunda*

Name	Conjugate	Extent Of Labeling	Form	Cat. No.
Lectin from <i>Wisteria floribunda</i>	biotin conjugate	-	lyophilized powder	L1516-.5MG L1516-2MG
Lectin from <i>Wisteria floribunda</i> , reduced form	biotin conjugate	biotin ~ 4 mol per 1 mol	lyophilized powder	L1766-.5MG L1766-1MG
Lectin from <i>Wisteria floribunda</i>	-	-	lyophilized powder	L8258-1MG L8258-5MG

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## Glycoprotein Purification

Purification of glycoproteins by selectively capturing the glycan component is commonly done utilizing affinity chromatography. The most common affinity matrices are *m*-aminophenylboronic acid-agarose for nonspecific binding of saccharides, and immobilized lectins for binding specific carbohydrates.

### *m*-Aminophenylboronic Acid Matrices

*m*-Aminophenylboronic acid matrices are capable of forming temporary bonds with any molecule that contains a 1,2-cis-diol group.

#### Procedure

Note: In general, equilibration buffers should be of low ionic strength, with pH 7-9. Equilibrate the column with buffer prior to use.

1. For a column volume of 1 mL, apply 1-2 mg of protein in ~250  $\mu$ L of buffer (50 mM taurine, adjusted to pH 8.7 with NaOH, containing 20 mM MgCl<sub>2</sub>).
2. Wash the column at a flow rate of 2 mL/hour, collecting 2 mL fractions until wash is free of protein.

3. Elute the bound protein using the same buffer (50 mM taurine, adjusted to pH 8.7 with NaOH, containing 20 mM MgCl<sub>2</sub>) with 50 mM sorbitol or 50 mM Tris-HCl added.

#### References:

1. Mallia, A.K. et al., Preparation and use of a boronic acid affinity support for separation and quantitation of glycosylated hemoglobins. *Anal. Letters*, **14**, 649-661 (1981).
2. *Immobilized Affinity Ligand Techniques*, Hermanson, G.T., et al., (eds.), (Academic Press, 1992), pp. 338, 339-392.
3. *Affinity Chromatography: A Practical Approach*, Dean, P.D.G., et al., (eds.), (IRL Press, 1985), p. 133.

### Glycoprotein Purification

Name	Matrix	Activation	Attachment	Spacer (atoms)	Extent Of Labeling	Peroxidase Type VI Binding Capacity (mg/mL)	Form	Cat. No.
<i>m</i> -Aminophenylboronic acid-Agarose	cross-linked 6% beaded agarose	epoxy	amino	12	5 - 20 $\mu$ mol per 1 mL	-	Suspension in 0.5 M NaCl, 0.1 M sodium acetate, pH 5.0.	A8530-5ML
<i>m</i> -Aminophenylboronic acid-Agarose	6% beaded agarose	epichlorohydrin	through amino to carboxyls of EDTA	9	40 - 80 $\mu$ mol per 1 mL	8 - 14	Suspension in water containing 0.002% chlorhexidine diacetate.	A8312-5ML

### Lectin Matrices

For a complete list of **Lectins** available, see page 52.

Immobilized lectins are used in affinity chromatography to selectively capture glycoproteins and glycopeptides.<sup>1</sup> The primary lectins used are Concanavalin A (Con A), wheat germ (*Triticum vulgare*), and jacalin (*Artocarpus integrifolia*), but additional lectin-agarose conjugates are available for specificity to less common glycans.

### Conditions for Lectin Affinity Chromatography

Lectin	Specificity	Equilibration/Wash Buffer	Elution Solution	References
<i>Arachis hypogaea</i> (Peanut) lectin; PNA	PNA binds specifically to Gal- $\beta$ (1 $\rightarrow$ 3)GalNAc-linkages found in O-linked glycoproteins. Immobilized lectin is used for the purification of O-linked glycoproteins and is specific for asialoglycoproteins.	10 mM sodium phosphate, 150 mM NaCl (PBS)	Equilibration buffer containing 0.1 M lactose	2
Concanavalin A (Con A, from <i>Canavalia ensiformis</i> )	Concanavalin A binds specifically to mannosyl and glucosyl residues of polysaccharides and glycoproteins. Unmodified hydroxyl groups at the C3, C4, and C6 positions of $\alpha$ -glucopyranosyl or $\alpha$ -mannopyranosyl rings may be essential for binding. Con A matrices have been used with SDS (0.05%) and TRITON <sup>®</sup> X-100.	1 M NaCl, 5 mM MgCl <sub>2</sub> , 5 mM MnCl <sub>2</sub> , and 5 mM CaCl <sub>2</sub>	Methyl $\alpha$ -D-glucopyranoside, methyl $\alpha$ -D-mannopyranoside, glucose, or mannose (concentration 5 mM to 500 mM)	3-5
<i>Galanthus nivalis</i> (Snowdrop) lectin; GNL	<i>Galanthus nivalis</i> lectin specificity is directed to the nonreducing end of the terminal $\alpha$ -D-mannosyl residue of glycoconjugates. Immobilized lectin has affinity for high mannose N-glycans.	10 mM sodium phosphate, 150 mM NaCl (PBS)	Equilibration buffer containing 0.5 M methyl $\alpha$ -D-mannopyranoside	6,7
Jacalin (lectin from <i>Artocarpus integrifolia</i> )	Used for the purification of glycoproteins containing $\alpha$ -D-galactose. Jacalin is specific toward the Thomsen-Friedenreich (T) antigen, but has subtle differences in its combining site when compared to the site of <i>Arachis hypogaea</i> (peanut) agglutinin.	100 mM Tris, pH 7.0, with 500 mM NaCl	Equilibration buffer containing either 20 mM methyl $\alpha$ -D-galactopyranoside or 100 mM melibiose	8-10
<i>Lens culinaris</i> (Lentil) lectin; LcH	Lentil lectin has an affinity for terminal $\alpha$ -D-mannosyl and $\alpha$ -D-glucosyl residues. The immobilized lectin binds selected bi- and triantennary complex N-glycans that are fucosylated.	10 mM sodium phosphate, 0.15 M NaCl (PBS), pH 7.3	Equilibration buffer containing 100 mM methyl $\alpha$ -D-mannopyranoside	11,12
<i>Triticum vulgare</i> (Wheat) lectin; WGA	Wheat lectin has an affinity for N-acetyl- $\beta$ -D-glucosaminyl residues and N-acetyl- $\beta$ -D-glucosamine oligomers. Immobilized wheat germ lectin is specific for GlcNAc <sub>2</sub> or NeuNAc residues.	0.05 M sodium phosphate, pH 7.0, containing 0.2 M NaCl	Equilibration buffer containing 100 mg/mL N-acetyl-D-glucosamine.	13,14

# Lectin Matrices

## Procedure for Lectin Affinity Chromatography

1. Centrifuge the agarose suspension for 30 seconds at 1,000 x g to pellet the resin. Discard the supernatant prior to equilibration.

### Equilibration and Binding

- Pre-wash and equilibrate the column with 5 column volumes of equilibration / wash buffer.
- Load sample solution in equilibration buffer (protein concentrations 1-20 mg/ml, free of particulates).
- Wash the resin with equilibration buffer until eluent is protein free.

### Elution

5. Elute the target protein with gradient or step-wise elution using elution solution.

### Additional Notes

a) Concanavalin A matrices: The general pH range is 6.5-7.5, although buffers as low as pH 4.1 and as high as 9.0 have been used successfully. A commonly used starting buffer is 20 mM Tris, pH 7.4, containing 0.5 M NaCl.

b) Concanavalin A matrices: Maximal recovery and cleaning of the resin may be achieved by using 1 M sucrose, glucose, mannose, or the corresponding  $\alpha$ -methyl glycoside. The addition of chaotropic agents (0.5 M to 6 M) may also be required for maximal recovery, but these denaturing conditions may severely damage the resin and therefore should be used as a last option.

c) Jacalin matrix: Jacalin-agarose has been reported to bind IgA. The loading buffer was 10 mM phosphate, pH 7.2 with 150 mM NaCl. Elution of bound IgA was performed with 800 mM D-galactose.

### References:

- Cummings, R.D., Use of lectins in analysis of glycoconjugates. *Meth. Enzymol.*, **230**, 66-86 (1994).
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- Shibuya, N., et al., Binding properties of a mannose-specific lectin from the snowdrop (*Galanthus nivalis*) bulb. *J. Biol. Chem.*, **263**, 728-734 (1988).
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- Roque-Barreira, M.C., and Campos-Neto, A., Jacalin: An IgA-binding lectin. *J. Immunol.*, **134**, 1740, (1985).
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## Lectins

Name	Matrix	Activation	Attachment	Spacer (atom)	Extent Of Labeling	Binding Capacity (mg/mL)	Form	Cat. No.
Lectin from <i>Arachis hypogaea</i> (peanut)	cross-linked 4% beaded agarose	cyanogen bromide	amino	1	2 - 4 mg/mL	asialofetuin ~1.5	Suspension in 1.0 M NaCl containing 0.01% thimerosal	L2507-1ML L2507-2ML
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean)	4% beaded agarose	p-nitrophenyl chloroformate	-	1	~ 10 mg/mL	yeast mannan 3 - 6	Suspension in 0.1 M acetate buffer, pH 6.0, containing 1 M NaCl, 1 mM Mn <sup>2+</sup> , 1 mM Ca <sup>2+</sup> , 1 mM Mg <sup>2+</sup> and 0.01% thimerosal	C6904-5ML C6904-10ML
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean)	cross-linked 4% beaded agarose	cyanogen bromide	amino	1	concanavalin A protein 15 - 30 mg/mL	yeast mannan 5 - 15	Suspension in 1.0 M NaCl + 0.1 M potassium phosphate, pH 6.0, with 1 mM CaCl <sub>2</sub> , 1 mM MgCl <sub>2</sub> and 1 mM MnCl <sub>2</sub> containing 0.02% thimerosal	C7555-5ML C7555-25ML C7555-100ML
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean)	Sepharose 4B	cyanogen bromide	amino	1	10 - 15 mg/mL	thyroglobulin 20 - 45	Suspension in 0.1 M acetate buffer, pH 6.0, containing 1 M NaCl, and 1 mM each of CaCl <sub>2</sub> , MgCl <sub>2</sub> , and MnCl <sub>2</sub>	C9017-25ML C9017-100ML
Lectin from <i>Galanthus nivalis</i> (snowdrop)	cross-linked 4% beaded agarose	-	-	-	protein 2 - 4 mg/mL	yeast mannan 5 - 10	Suspension in 1.0 M NaCl containing 0.01% thimerosal	L8775-1ML L8775-2ML
Lectin from <i>Artocarpus integrifolia</i>	cross-linked 4% beaded agarose	-	-	-	~ 5 mg/mL	human IgA 1 - 2	Suspension in 1 M NaCl containing 0.01% thimerosal	L5147-2ML L5147-5ML
Lectin from <i>Lens culinaris</i> (lentil)	4% beaded agarose	cyanogen bromide	-	1	2 - 4 mg/mL	-	Suspension in 0.9% NaCl, 1 mM CaCl <sub>2</sub> , 1 mM MnCl <sub>2</sub> containing 0.01% thimerosal	L4018-1ML L4018-5ML
Lectin from <i>Lens culinaris</i> (lentil)	Sepharose 4B	-	-	-	~ 2 mg/mL	porcine thyroglobulin 15	Suspension in 20% ethanol containing 0.9% NaCl, 1 mM CaCl <sub>2</sub> , 1 mM MnCl <sub>2</sub> and 0.01% thimerosal	L0511-5ML L0511-10ML L0511-25ML
Lectin from <i>Triticum vulgare</i> (wheat)	6% agarose macrobeads	cyanogen bromide	-	1	~ 6 mg/mL	ovomucoid 1 - 2	Suspension in 0.9% NaCl containing 0.01% thimerosal	L1394-1ML L1394-5ML L1394-10ML
Lectin from <i>Triticum vulgare</i> (wheat)	cross-linked 4% beaded agarose	cyanogen bromide	-	-	5 - 10 mg/mL	-	Suspension in 1.0 M NaCl containing 0.02% thimerosal	L1882-1ML L1882-5ML

## Glycoprotein Detection

Initial detection of glycoproteins *in vitro* is routinely accomplished on SDS-PAGE gels and Western Blots. Cellular localization of glycoproteins is normally accomplished utilizing lectin fluorescent conjugates. For a complete list of **Lectins**, including fluorescently labeled lectins, see page 52.

### Fluorescent Detection on SDS-PAGE Gels

#### GlycoProfile™ III Fluorescent Glycoprotein Detection Kit

Identify glycoproteins with superior selectivity using Sigma's new GlycoProfile III Detection Kit. Allows fluorescent detection of glycoproteins directly in PAGE gels without blotting or use of antibodies. ProteoProfile™ PTM Marker (Cat. No. P1745) contains a mix of glycosylated, phosphorylated, and unmodified proteins and is available individually as well as with GlycoProfile III.

#### Features and Benefits

- Superior selectivity—enables more accurate detection of glycoproteins
- Excellent sensitivity—allows you to detect 150 ng of glycoprotein (5 to 30 ng carbohydrate)
- Compatibility with both PAGE gels and PVDF membranes—allows flexibility in your applications



Figure 1. Components of GlycoProfile III Fluorescent Glycoprotein Staining Kit (Cat. No. PP0300).

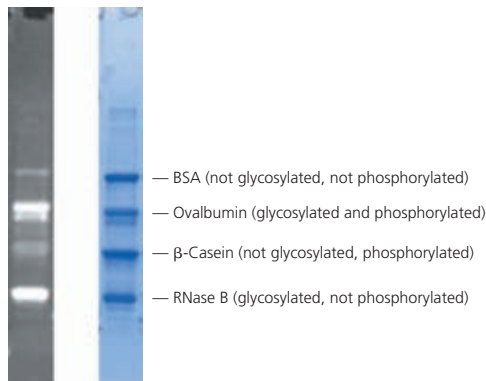


Figure 2. PTM Marker (2 μl of a 6-fold dilution), containing glycosylated and non-glycosylated proteins, was separated by electrophoresis on a 4–20% SDS-PAGE gel. The gel was stained for glycoproteins with GlycoProfile III (left), imaged, and then stained for total protein with EZBlue™ Gel Staining Reagent (right). The glycoproteins appear as bright fluorescent bands. Each band represents approximately 300 ng of protein.

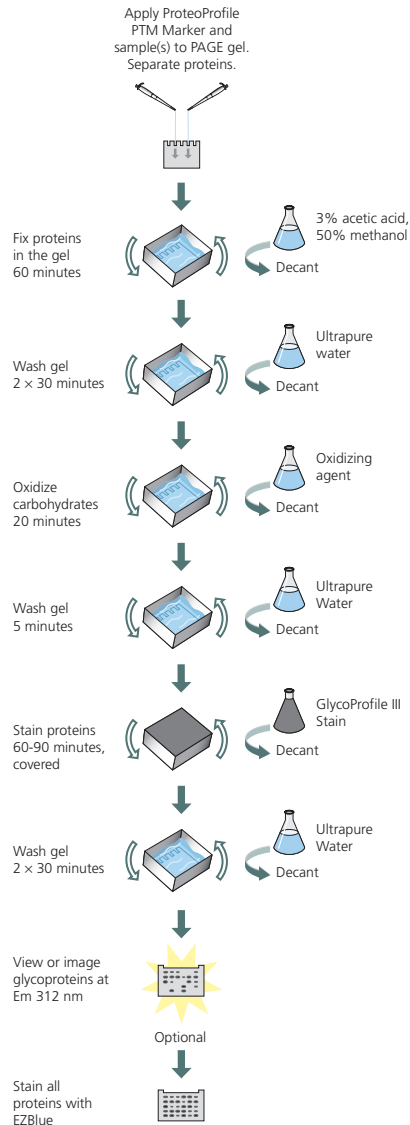


Figure 3. Schematic of staining procedure for GlycoProfile III Fluorescent Staining Kit.

#### GlycoProfile™ III, Fluorescent Glycoprotein Detection Kit

Allows for the specific, sensitive fluorescent detection of the glycoproteins directly in gels. This kit can also be used to detect glycoproteins after transfer to PVDF membranes.

#### Components

- Glycoprotein Staining Reagent for GlycoProfile™ III
  - Oxidation Reagent for GlycoProfile™ III
  - Staining Buffer for GlycoProfile™ III
  - ProteoProfile™ PTM Marker (Sigma P1745)
- store at: 2–8°C

PP0300-1KT	1 kit
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## Fluorescent Detection on SDS-PAGE Gels

### EZBlue™ Gel Staining Reagent

A ready-to-use Brilliant Blue G-250 based protein stain for "one step" ultrasensitive detection on polyacrylamide gels and PVDF membranes. store at: 2-8°C

G1041-500ML	500 mL
G1041-3.8L	3.8 L

### ProteoProfile™ PTM Marker

The protein post-translational modifications (PTM) of glycosylation and phosphorylation are encountered frequently in proteomic analysis. ProteoProfile™ PTM Marker, containing glycosylated and phosphorylated proteins, is designed for use as a positive and negative control in studying these post-translation modifications of proteins in SDS-PAGE. Electrophoresis will yield four protein bands:

- Albumin (no modification)
- Ovalbumin (both phosphorylated and glycosylated)
- $\beta$ -casein (phosphorylated)
- RNase B (glycosylated)

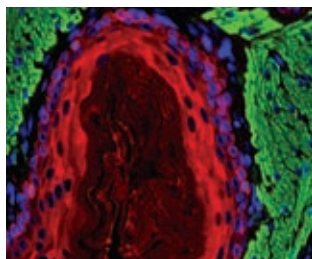
store at: room temp

P1745-100UL

## Lectins for the Fluorescent Detection of Glycoproteins

Name	Conjugate	Application	Form	Cat. No.
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean)	FITC conjugate	For the fluorescent detection of glycoproteins containing $\alpha$ -D-mannose or $\alpha$ -D-glucose	lyophilized powder	C7642-2MG C7642-10MG
Lectin from <i>Triticum vulgare</i> (wheat)	FITC conjugate	For the fluorescent detection of glycoproteins containing $\beta$ (1 $\rightarrow$ 4)-N-acetyl-D-glucosamine	lyophilized powder	L4895-2MG L4895-5MG L4895-10MG

## Detection Web Resource



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# Detection of Biotin Labeled Glycoproteins on Western Blots

## Detection of Biotin Labeled Glycoproteins on Western Blots

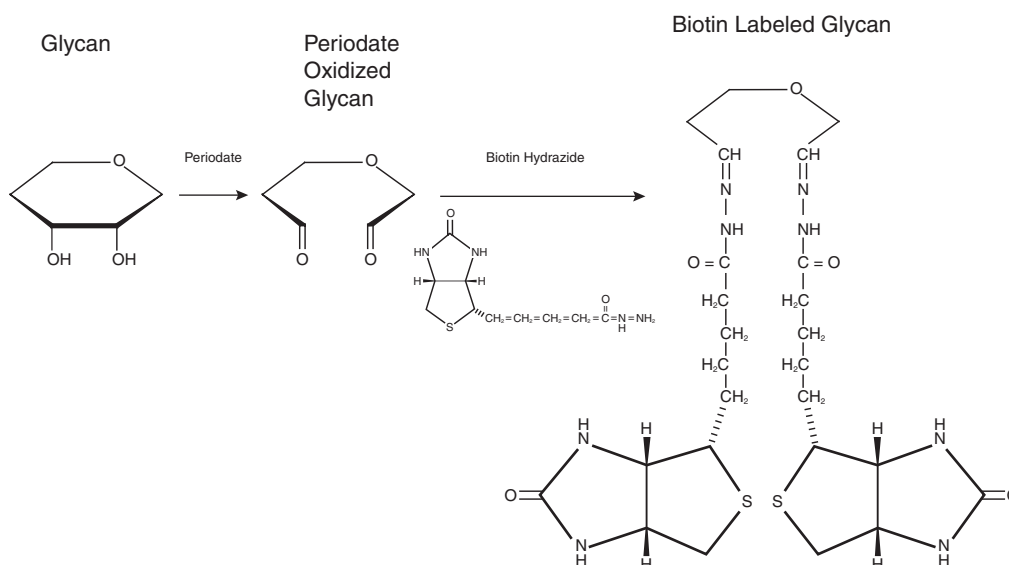
Biotin-hydrazide modification of oxidized glycans (see **Figure 4**) can be utilized to label glycoproteins prior to electrophoresis or after Western blotting.<sup>1</sup>

Carbohydrate residues in solutions of glycoproteins are chemically oxidized by incubation for 30 minutes using sodium periodate (final concentration 1 mM for oxidation of terminal sialic acid residues or 10 mM for oxidation of general sugar moieties). After reaction, any excess periodate is removed by dialysis against phosphate buffered saline. The glycoprotein is subsequently incubated for 2 hours with a 5 mM solution of biocytin hydrazide or biotin hydrazide to attach biotin to the oxidized glycan sites. After reaction and dialysis, the biotin-labeled glycoprotein may be applied to an electrophoresis gel.

Glycoproteins on blots may also be labeled using biocytin hydrazide. Bayer, et al., reported that biocytin hydrazide, but not biotin hydrazide, is suitable for labeling of blotted glycoconjugates.<sup>1</sup> The procedure is similar to that used for glycoproteins in solution. The Western blot must be blocked with an appropriate protein prior to incubation with sodium periodate solution (1 mM or 10 mM) for 30 minutes. After incubation, the blot is then rinsed thoroughly and incubated with 3 µg/mL biocytin hydrazide for 1 hour.

Enzymatic oxidation of carbohydrates can be performed using neuraminidase to oxidize terminal sialic acid groups and galactose oxidase to oxidize terminal galactose groups. The enzyme solution(s) and biocytin hydrazide are both included in the reaction for simultaneous, rather than sequential, labeling. Solutions of glycoproteins (0.5-3 mg/ml) and glycoproteins immobilized on Western blots may be labeled by enzymatic means. Refer to References 1 and 2 for more complete procedural details.

After electrophoresis and Western transfer, biotin-conjugated glycoproteins can be probed with streptavidin-peroxidase. Detection can be accomplished with either a colorimetric tetramethylbenzidine (TMB) or chemiluminescent peroxidase substrate. Alkaline phosphatase-conjugated streptavidin can also be used to probe the blots with subsequent detection using a colorimetric BCIP/NBT or CDP-Star<sup>®</sup> Chemiluminescent Substrate. Typical detection limits for glycoproteins using the streptavidin-peroxidase method are ~ 50-100 ng. Optimal results are obtained on PVDF membranes.



**Figure 4.** Schematic of biotin labeling reaction to periodate oxidized glycan.

### References:

1. Bayer, E.A., et al., Analysis of proteins and glycoproteins on blots. *Meth. Enzymol.*, **184**, 415-427 (1990).
2. Bayer, E.A., et al., Biocytin hydrazide—a selective label for sialic acids, galactose, and other sugars in glycoconjugates using avidin-biotin technology. *Anal. Biochem.*, **170**, 271-281 (1988).

# Detection of Biotin Labeled Glycoproteins on Western Blots

## Products for Biotin Labeling of Glycoproteins and Western Blot Detection

Name	Application	Cat. No.
Biocytin hydrazide	Reactive derivative for labeling proteins and glycoproteins with biotin.	B9014-10MG
(+)-Biotinamidohexanoic acid hydrazide	Biotinylation reagent with aminocaproic spacer for aldehyde groups (e.g., periodate-oxidized sugars) and carboxylic acids. Typically used for coupling to glycoproteins through the carbohydrate by hydrazone bond formation.	B3770-25MG B3770-100MG B3770-250MG
(+)-Biotin hydrazide	Typically used for coupling to glycoproteins through the carbohydrate by hydrazone formation.	B7639-100MG B7639-1G
Chemiluminescent Peroxidase Substrate-1	Two component liquid peroxidase substrate system for Western blot detection. Picomole detection with superior signal-to-noise ratio. Component ratios can be adjusted for optimal signal duration, sensitivity and background.	CPS160-1KT
Chemiluminescent Peroxidase Substrate-1		CPS1120-1KT
Chemiluminescent Peroxidase Substrate-1		CPS1300-1KT
Chemiluminescent Peroxidase Substrate-3	CPS3 formulations are economically priced and outperform the most commonly used competitors' products. This two component system provides low nanogram detection with superior signal-to-noise ratio. In addition, the components are stable after mixing, adding to the flexibility of usage.	CPS350-1KT
Chemiluminescent Peroxidase Substrate-3		CPS3500-1KT
Galactose Oxidase from <i>Dactylium dendroides</i>	Useful in the determination of lactose.	G7907-150UN G7907-450UN
Neuraminidase from <i>Vibrio cholerae</i>	Neuraminidase is used as a cell-surface probe for glycoconjugate distribution and in substrate specificity studies.	N7885-1UN N7885-2UN
Sodium periodate	Generates quinones <i>via</i> glycol cleavage and oxidation of hydroquinones.	311448-5G 311448-100G 311448-500G
Streptavidin-Alkaline Phosphatase from <i>Streptomyces avidinii</i>	The working dilution should be determined using a range of dilutions from a 1 mg/mL stock in buffer.	S2890-250UG S2890-1MG
Streptavidin-Peroxidase from <i>Streptomyces avidinii</i>	The working dilution should be determined using a range of dilutions from a 1 mg/ml stock in buffer.	S5512-.1MG S5512-250UG S5512-.5MG S5512-1MG S5512-2MG
3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System for Membranes	TMB is a substrate for horseradish peroxidase. Develops a permanent, insoluble, dark blue reaction product. Recommended for membrane applications, not recommended for ELISA (microwell) procedures.	T0565-100ML

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## Colorimetric Detection on PAGE and Western Blots

### Glycoprotein Detection Kit

Sigma's colorimetric glycoprotein detection kit enables convenient, reliable detection of the sugar moieties of glycoproteins in PAGE gels or Western blotting membranes. Based on a modification of Periodic acid-Schiff (PAS) methods, the Glycoprotein Detection Kit identifies glycoproteins with magenta bands on a light pink or colorless background. The kit contains reagents sufficient to stain ten mini-gels (10 × 10 cm) or ten membranes of the same dimensions and detects as little as 25 to 100 ng of carbohydrate, depending on the nature and degree of glycosylation.



Figure 6. Components of Glycoprotein Detection Kit (Cat. No. GLYCOPRO).

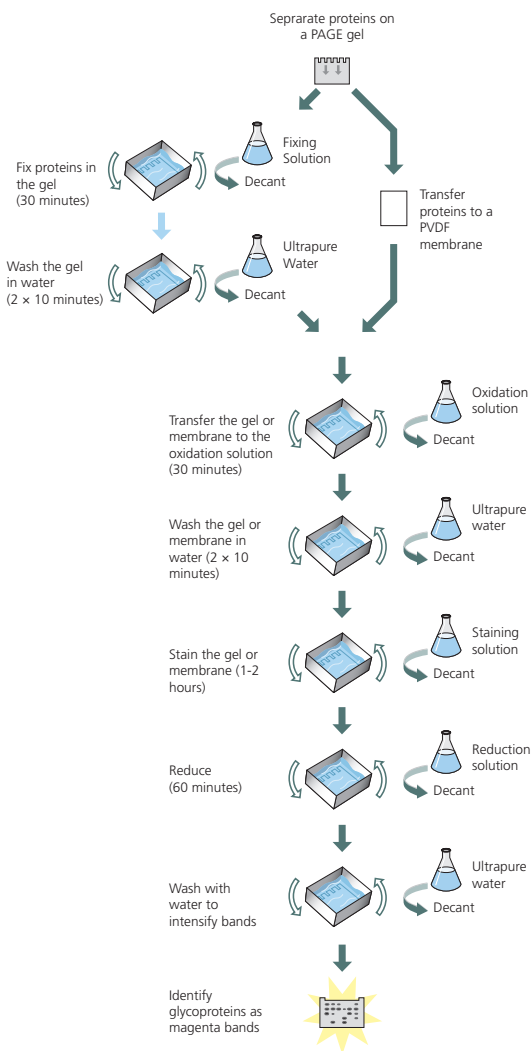


Figure 5. Schematic of Glycoprotein Detection Kit staining procedure.

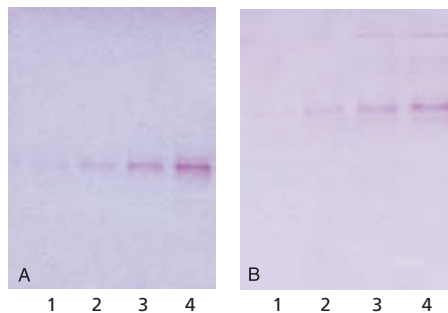
### Glycoprotein Detection Kit

1 kit sufficient for 10 mini-gels (10 × 10 cm)  
1 kit sufficient for 5 gels or membranes (16.5 × 19 cm)

#### Components

- Horseradish Peroxidase (Positive control)
- Oxidation component (Periodic Acid)
- Reduction component (Sodium Metabisulfite)
- Schiff's reagent, Fuchsin-sulfite reagent

**Lit. cited:** 1. Zacharius, R.M., et al., *Anal. Biochem.* **31**, 148-152 (1969)  
2. Thornton, D.J., et al., Walker, J.M., ed., *Methods Mol. Biol.*, Humana Press (Totowa, NJ: 1994), **32**, 119-128  
store at: 2-8°C



Peroxidase was run on a 10% SDS-PAGE gel (panel A) and also transferred to PVDF (panel B). Lane 1 - 0.125 µg, lane 2 - 0.25 µg, lane 3 - 0.5 µg, lane 4 - 1 µg. The amount of carbohydrate loaded represents approx. 16% of the total protein.

GLYCOPRO-1KT 1 kit

# Colorimetric Detection on PAGE and Western Blots

## Lectins and Other Products for the Colorimetric or Chemiluminescent Detection of Glycoproteins

Name	Conjugate	Application	Cat. No.
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean)	biotin conjugate	Detects glycoproteins containing $\alpha$ -D-mannose, $\alpha$ -D-glucose when used with avidin or streptavidin detection system.	C2272-2MG C2272-10MG
lyophilized powder			
Concanavalin A from <i>Canavalia ensiformis</i> (Jack bean)	peroxidase conjugate	Detects glycoproteins containing $\alpha$ -D-mannose, $\alpha$ -D-glucose when used with appropriate peroxidase substrate	L6397-1MG L6397-5MG
lyophilized powder			
IgM, Lambda from murine myeloma buffered aqueous solution	-	-	M5170-1MG
Lectin from <i>Triticum vulgare</i> (wheat)	biotin conjugate	Detects glycoproteins containing $\beta$ (1 $\rightarrow$ 4)-N-acetyl-D-glucosamine when used with avidin or streptavidin detection system	L5142-1MG L5142-5MG
lyophilized powder			
Lectin from <i>Triticum vulgare</i> (wheat)	peroxidase conjugate	Detects glycoproteins containing $\beta$ (1 $\rightarrow$ 4)-N-acetyl-D-glucosamine when used with appropriate peroxidase substrate	L3892-1MG L3892-2MG L3892-5MG
lyophilized powder			
Periodic acid	-	Periodic acid is commonly used as a component for glycoprotein stains. Selective staining of glycoproteins on SDS-PAGE and membranes is carried out using a modification of the Periodic Acid-Schiff (PAS) method. Staining of sugar moieties of glycoproteins yields magenta bands with a colorless background. The Periodic Acid/Schiff Reagent stains vicinal diol groups found mainly on peripheral sugars and sialic acids and is used as a general glycoprotein stain. Periodic acid is also used to cleave reversible cross-linkers (e.g. DATD, DHEBA) in PAGE gels.	P0430-25G P0430-100G
Schiff's fuchsin-sulfite reagent	-	Useful for the detection of glycoproteins in polyacrylamide gels.	S5133-500ML S5133-1L

## Glycoprotein Standards

There are many methods utilized in glycoprotein detection and identification, with the most common being SDS-PAGE, mass spectroscopy, HPLC, NMR and Western blotting. It is helpful and often necessary to include glycoproteins as standards and/or molecular weight markers. In addition to these specific glycoprotein standards, Sigma offers a broad variety of glycoproteins for analytical and biological use as controls and bioactive components. See **Glycoproteins** and **Neoglycoproteins** on pages 115 and 117 for further listings.

### Invertase Glycoprotein Standard, Proteomics Grade

Invertase is an enzyme that catalyses the hydrolysis of sucrose into fructose and glucose. The Invertase Glycoprotein Standard is the periplasmic (glycosylated form, external invertase) with 50% of its mass as polymannan. The periplasmic invertase molecule can exist in a number of association states, each a multiple of the core glycosylated monomer, a 60 kDa peptide plus oligosaccharide chains. Depending on the extraction, purification and storage conditions, invertase will exist as a dimer, tetramer, hexamer, or octamer. Since yeast provides a system for protein glycosylation similar to mammalian systems, periplasmic invertase is often used as a model glycoprotein. The Invertase Glycoprotein Standard can be used to demonstrate N-glycosylation using PNGase F with both in-solution and in-gel procedures. The extent of deglycosylation can be assessed by mobility shift on SDS-PAGE gels.

### RNase B Glycoprotein Standard, Proteomics Grade

Bovine pancreatic Ribonuclease B (RNase B) is a glycoprotein that contains only N-linked glycans. It is a globular protein composed of a single domain that occurs naturally as a lesser component in a mixture along with Ribonuclease A (RNase A), which is the non-glycosylated form. RNase B contains a single glycosylation site at Asn<sup>34</sup> at which five to nine mannose residues are attached to the chitobiose core, i.e. Man<sub>5</sub>GlcNAc<sub>2</sub>, Man<sub>6</sub>GlcNAc<sub>2</sub>, Man<sub>7</sub>GlcNAc<sub>2</sub>, Man<sub>8</sub>GlcNAc<sub>2</sub> and Man<sub>9</sub>GlcNAc<sub>2</sub>. Due to the heterogeneity in the glycosylation at Asn<sup>34</sup>, RNase B exists as five glycosylated variants, with a molecular mass of approximately 15 kDa. RNase B Glycoprotein Standard has been highly purified to remove contaminating RNase A.

RNase B is a preferred substrate with PNGase F for demonstration of N-linked deglycosylation using SDS-PAGE or MALDI-MS. The activity of PNGase F is routinely assayed using RNase B by monitoring the pronounced mobility shift in 12% gels after deglycosylation. Proteomics Grade RNase B can also be used as a source of N-glycans following enzymatic digestions and subsequent purification.

### Invertase Glycoprotein Standard

Invertase;  $\beta$ -D-Fructofuranosidase;  $\beta$ -D-Fructofuranoside fructohydrolase; Saccharase  
[9001-57-4] E.C. 3.2.1.26

#### ► Proteomics Grade, from *Saccharomyces cerevisiae*

≥0.5 mg/vial protein (Bradford)

lyophilized powder

store at: 2-8°C

I0408-500UG	500 µg
-------------	--------

### RNase B Glycoprotein Standard from bovine pancreas

Ribonuclease B; RNase B  
[9001-99-4]

#### ► Proteomics Grade

≥90% (SDS-PAGE)

lyophilized powder

store at: -20°C

R1153-500UG	500 µg
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R1153-1MG	1 mg
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## Deglycosylation Strategies

The removal of glycans from glycosylated proteins or other molecules is often a precursor to analysis of the glycan structures. For glycoproteins, both selective and nonselective glycosylation methods may be applied to determine the structure of the protein core and its glycosylation sites. The complexity of a protein population that results from glycosylation can be reduced by enzymatic or chemical deglycosylation. Enzymatic methods are relatively mild and allow removal of a selected class of glycans without protein or glycan degradation, although complete removal of glycans often requires denaturation of the protein. Chemical deglycosylation methods are less selective than enzymatic techniques. Depending upon the method used, either the protein core or the glycans can be recovered intact without significant degradation.

Removal of glycans from a glycoprotein is used:

- To simplify analysis of the peptide portion of the glycoprotein
- To simplify the analysis of the glycan component
- To remove heterogeneity in glycoproteins for X-ray crystallographic analysis
- To remove carbohydrate epitopes from antigens
- To investigate the role of carbohydrates in enzyme activity and solubility
- To investigate ligand binding
- For quality control of glycoprotein pharmaceuticals

## Chemical Deglycosylation

### Hydrazinolysis

Hydrazine hydrolysis has been found to be effective in the complete release of unreduced O- and N-linked oligosaccharides. Selective and sequential release of oligosaccharides can be accomplished by mild hydrazinolysis of the O-linked oligosaccharides at 60 °C initially, followed by N-linked oligosaccharides at 95 °C. Hydrazine hydrolysis leaves the glycan intact but results in destruction of the protein component (see **Figure 1**).

Complete details for this procedure are given in *Methods in Enzymology*.<sup>1</sup> Care should be taken during all steps of this procedure as many of the reagents and reactions are extremely hazardous and highly reactive.

Glycan release is accomplished by the addition of fresh anhydrous hydrazine to a salt-free, freshly lyophilized glycoprotein sample. The volume of hydrazine should produce a protein concentration of 5 to 25 mg/ml. The reaction mixture should be capped immediately. For the release of both N- and O-linked glycans, incubation should be for 4 hours at 95 °C. For the selective release of O-linked glycans, incubation at 60 °C for 5 hours is suitable.

Excess unreacted hydrazine can be removed under high vacuum at a temperature not exceeding 25 °C. The vacuum pump should be equipped with an activated charcoal/alumina trap. Addition of small aliquots of anhydrous toluene may be required to bring the sample to complete dryness.

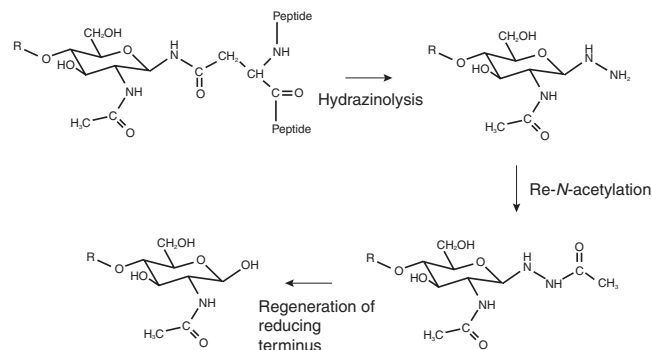
Acetyl groups attached to the carbohydrate through an amine may be removed by hydrazinolysis. These groups must be replaced to stabilize the reducing ends of the released oligosaccharides. The dried sample is re-N-acetylated while on ice by the addition of ice-cold saturated aqueous sodium bicarbonate, followed immediately by the addition of acetic anhydride. The sample is mixed gently and incubated at room temperature for 10 minutes. A second aliquot of acetic anhydride equal in volume to the first is added to the reaction and incubated for an additional 20 minutes. The volume of the two aliquots should result in a 5x molar excess of acetic anhydride over the amine content of the protein. The volume of sodium bicarbonate added should yield a final concentration of 0.5 M acetic anhydride in the reaction mixture.

A small amount of the released glycan pool may exist as the aceto-hydrazide derivative. These derivatives may be converted to the unreduced glycans by resuspension of the dried glycan pool in 1 mM Cu (II) acetate in 1 mM acetic acid and incubation at room temperature for one hour.

Dowex® 50Wx2 can be used to remove the excess cations by washing the sample through the resin with water.

Glycan and degraded protein components can be separated by gel filtration or by paper chromatography.

Note: Use of hydrazinolysis for release and isolation of glycans is covered by U.S. patents 5,539,090 and 6,180,779, and their foreign equivalents.



**Figure 1.** Removal of glycans from glycoproteins by hydrazinolysis. Free glycans must be re-N-acetylated and then reduced to remove hydrazide group.

### References:

1. Patel, T.P. and Parekh, R.B., Release of oligosaccharides from glycoproteins by hydrazinolysis. *Meth. Enzymol.*, **230**, 57-66 (1994).
2. Patel, T., et al., Use of hydrazine to release in intact and unreduced form both N- and O-linked oligosaccharides from glycoproteins. *Biochemistry*, **32**, 679-693 (1993).
3. Bendiak, B., and Cumming, D.A., Purification of oligosaccharides having a free reducing-end from glycopeptide sources. *Carbohydr. Res.*, **151**, 89-103 (1986).
4. Makino, Y., et al., Structural analysis of N-linked sugar chains of human blood clotting factor IX. *J. Biochem. (Tokyo)*, **128**, 175-180 (2000).
5. Takasaki, S., et al., Hydrazinolysis of asparagine-linked sugar chains to produce free oligosaccharides. *Meth. Enzymol.*, **83**, 263-8 (1982).

# Chemical Deglycosylation

Name	Assay (%)	Cat. No.
Hydrazine, anhydrous	98	215155-50G 215155-100G 215155-500G
Toluene, anhydrous	99.8	244511-100ML 244511-12X100ML 244511-1L 244511-6X1L 244511-2L 244511-4X2L 244511-18L 244511-56L 244511-200L
Dowex® 50Wx2 hydrogen form	-	217476-100G 217476-500G 217476-2.5KG
Copper(II) acetate monohydrate, ACS reagent	98.0-102.0 (ACS specification) ≥98	217557-100G 217557-500G 217557-2.5KG
Acetic anhydride, reagent grade	≥98	A6404-200ML A6404-500ML A6404-1L
Sodium bicarbonate, Reagent-Plus®	≥99.5	S8875-500G S8875-1KG S8875-2.5KG S8875-5KG
Toluene, anhydrous	99.8	244511-100ML 244511-12X100ML 244511-1L 244511-6X1L 244511-2L 244511-4X2L 244511-18L 244511-56L 244511-200L

## Alkaline β-Elimination

Release of glycans by alkaline β-elimination utilizes ammonium hydroxide/carbonate or sodium hydroxide in conjunction with sodium borohydride. Like hydrazinolysis, β-elimination results in total destruction of the protein backbone.<sup>1-3</sup>

O-Glycosidic linkages between glycans and the β-hydroxyl groups of serine or threonine are readily hydrolyzed by dilute alkaline solutions (0.05 to 0.1 M sodium hydroxide or potassium hydroxide) under mild conditions (45 to 60 °C for 8 to 16 hours) leading to the liberation of O-linked glycans by the mechanism of β-elimination. To prevent isomerization or degradation of the released carbohydrates by 'peeling' reactions, the hydrolysis is performed in the presence of a reducing agent (0.8 to 2 M sodium borohydride). This results in the formation of the reduced (alditol) forms of the glycans. N-Linked glycans and O-linked glycans that are attached to tyrosine, hydroxyproline and hydroxylysine are not cleaved under these conditions. Furthermore, the β-elimination reaction does not take place if the glycan is attached to serine or threonine at the carboxy-terminus position of the protein.

For quantitative release of N-linked glycans, harsher alkaline conditions are required (1 M sodium hydroxide at 100 °C for 6 to 12 hours). As for O-linked glycans, the reaction must be performed under reducing conditions (1 to 2 M sodium borohydride) to prevent 'peeling' reactions taking place on the released N-glycans. N-Acetylglucosamine (GlcNAc) residues are deacetylated during this reaction and must be re-N-acetylated by using acetic anhydride in methanol during the recovery of the glycans.

The primary limitation of alkaline β-elimination is the high residual sodium content after reaction. The high salt content interferes with downstream mass spectrometry techniques for glycan mapping and sequencing. An alternative method for β-elimination uses ammonia in place of hydroxide for the high pH conditions required to cleave the O-linked glycan residues. However, β-elimination using ammonia may be incomplete for some proteins.<sup>4</sup>

A 5 mg/ml solution of borate-ammonia complex in 28% aqueous ammonia is used to hydrolyze the O-glycosidic bonds. Glycoproteins are incubated at 45 °C for 18-24 hours with the borate-ammonia complex solution to release the O-linked glycans. Excess ammonia is removed by flushing the sample with nitrogen until dry. The sample is reconstituted with water and passed through a cation exchange resin such as Dowex® 50Wx8. Excess boric acid is removed by repeated reconstitution with methanol and evaporation using nitrogen. The released glycans can be redissolved in water and labeled for mass spectrometric analysis without desalting.<sup>5,6</sup>

### References:

- Carlson, D.M., and Blackwell, C., Structures and immunochemical properties of oligosaccharides isolated from pig submaxillary mucins. *J. Biol. Chem.*, **243**, 616-626 (1968).
- Lloyd, K.O., et al., Comparison of O-linked carbohydrate chains in MUC-1 mucin from normal breast epithelial cell lines and breast carcinoma cell lines. Demonstration of simpler and fewer glycan chains in tumor cells. *J. Biol. Chem.*, **272**, 33325-34 (1996).
- Morelle, W., et al., Structural analysis of oligosaccharide-alditols released by reductive β-elimination from oviducal mucins of *Rana dalmatina*. *Carbohydr. Res.*, **306**, 435-443 (1998).
- Tarelli, E., Resistance to deglycosylation by ammonia of IgA1 O-glycopeptides: implications for the β-elimination of O-glycans linked to serine and threonine. *Carbohydr. Res.*, **342**, 2322-5 (2007).
- Huang, Y., et al., Matrix-assisted laser desorption/ionization mass spectrometry compatible β-elimination of O-linked oligosaccharides. *Rapid Commun. Mass Spec.*, **16**, 1199-1204 (2002).
- Huang, Y., et al., Microscale nonreductive release of O-linked glycans for subsequent analysis through MALDI mass spectrometry and capillary electrophoresis. *Anal. Chem.*, **73**, 6063-6069 (2001).

Name	Assay (%)	Cat. No.
Sodium borohydride, Reagent-Plus®	99	213462-25G 213462-100G
Borane-ammonia complex, technical grade	90	287717-1G 287717-10G
Dowex® 50Wx8 hydrogen form	-	217514-1FT3 217514-100G 217514-500G 217514-2.5KG
Acetic anhydride, reagent grade	≥98	A6404-200ML A6404-500ML A6404-1L
Sodium hydroxide, reagent grade	≥98	S5881-500G S5881-1KG S5881-5KG
Borane-ammonia complex, technical grade	90	287717-1G 287717-10G
Sodium borohydride, Reagent-Plus®	99	213462-25G 213462-100G

## Trifluoromethanesulfonic acid

### GlycoProfile IV Chemical Deglycosylation Kit

The GlycoProfile Chemical Deglycosylation Kit has been optimized to provide a rapid (~1 hour), convenient, and reproducible method to remove glycans from glycoproteins by reaction with trifluoromethanesulfonic acid (TFMS). The deglycosylated protein can then be recovered using a suitable downstream processing method. The kit contains sufficient reagents and a glycoprotein standard, for a minimum of 10 reactions when the sample size is between 1 and 2 mg of a typical glycoprotein. Unlike other chemical deglycosylation methods, hydrolysis with anhydrous TFMS is very effective at removing O and N-linked glycans (except the innermost Asn-linked GlcNAc or GalNAc).

Trifluoromethanesulfonic acid (TFMS) hydrolysis leaves an intact protein component, but results in destruction of the glycan. Glycoproteins from animals, plants, fungi, and bacteria have been deglycosylated by this procedure. Comparisons of functional interactions before and after treatment allow analysis of biological, immunological, and receptor binding properties. This permits a more complete understanding of the processing and roles in different stages of the glycoprotein's lifecycle. The reaction is non-specific, removing all types of glycans, regardless of structure, although prolonged incubation is required for complete removal of O-linked glycans. Also, the innermost Asn-linked GlcNAc residue of N-linked glycans remains attached to the protein. The method removes the N-glycans of plant glycoproteins that are usually resistant to enzymatic hydrolysis.

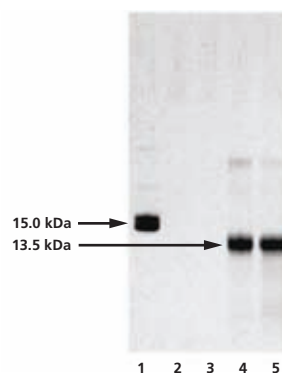
#### Features and Benefits

- Removes glycans from amino acids—permits sequence analysis of the protein by mass spectrometry, comparison DNA to protein sequence
- Eliminates glycans affecting molecular radius—allows molecular mass determination by electrophoresis
- Complete removal in single process—saves time by eliminating need for multiple enzyme reactions, overcoming enzyme resistance
- Complete kit—eliminates waste and handling of excess reagents

Contains reagents sufficient to deglycosylate up to 10 samples (1-2 mg each) of a typical glycoprotein or glycoprotein standard.



**Figure 2.** Components of GlycoProfile IV Chemical Deglycosylation Kit (Cat. No. PP0510).



**Figure 3.** Analysis of the chemical deglycosylation of RNase B on 12% homogeneous SDS-PAGE gel. Lane 1 is the RNase B control (Cat. No. R1153), while lanes 2 to 5 represent fractions collected from the gel filtration column. Lanes 2 and 3 are pre-void volume fractions and lanes 4 and 5 show bands at 13.5 kDa, corresponding to deglycosylated RNase B.

#### References:

1. Edge, A.S.B., Deglycosylation of glycoproteins with trifluoromethanesulfonic acid: elucidation of molecular structure and function. *Biochem. J.*, **376**, 339-350 (2003).
2. Edge, A.S.B., et al., Deglycosylation of glycoproteins by trifluoromethanesulfonic acid. *Anal. Biochem.*, **118**, 131-137 (1981).
3. Burgess, A. J., and Norman, R. I., The large glycoprotein subunit of the skeletal muscle voltage-sensitive calcium channel. Deglycosylation and development. *Eur. J. Biochem.* **178**, 527-533 (1988).
4. Sojar, H.T., and Bahl, O.P., Chemical deglycosylation of glycoproteins. *Meth. Enzymol.*, **138**, 341-350 (1987).

### GlycoProfile™ IV Chemical Deglycosylation Kit

#### ► TFMS Deglycosylation System

The kit contains sufficient reagents for 10 reactions with a sample size between 1-2 mg of a typical glycoprotein. An RNase B glycoprotein standard is included.

#### Components

Trifluoromethanesulfonic Acid (TFMS) 5 x 1 g  
 RNase B Glycoprotein Standard 3 x 1 mg  
 Pyridine solution, 60% 10 ml  
 Bromophenol blue solution, 0.2% 0.5 ml  
 Anisole, anhydrous 5 x 1 ml  
 Reaction vials 10 each  
 store at: 2-8°C

PP0510-1KT	1 kit
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# Enzymatic Deglycosylation

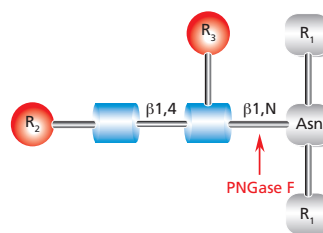
## Enzymatic Deglycosylation

Sequential hydrolysis of individual monosaccharides from glycans can be useful for the elucidation of the structure and function of the glycan component. Due to the restraints of the specificity of glycolytic enzymes currently available, sequential hydrolysis of individual monosaccharides is also required in many instances in order to completely remove a glycan component enzymatically. This is particularly true in the enzymatic deglycosylation of many O-linked glycans. Both endoglycosidases and exoglycosidases are used for deglycosylation procedures. Endoglycosidases are enzymes that catalyze the cleavage of an internal glycoside bond in an oligosaccharide. Exoglycosidases are enzymes that remove terminal carbohydrates from the non-reducing end of a glycan, but do not cleave internal bonds between carbohydrates.

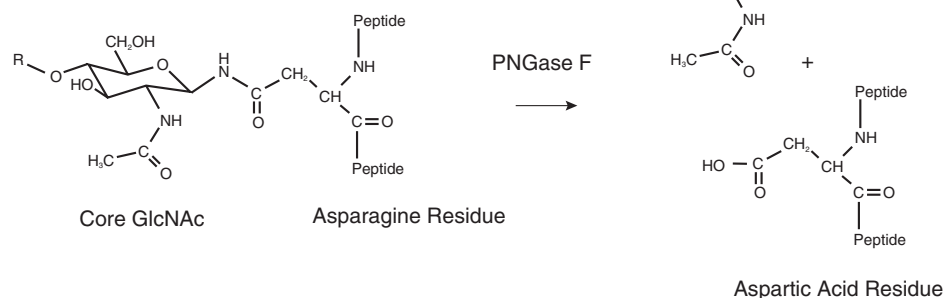
See **Glycan Sequencing using Exoglycosidases** on page 85 for discussion of exoglycosidases; **Exoglycosidases** available from Sigma are listed on page 91.

## N-Linked Glycan Strategies

Use of the endoglycosidic enzyme PNGase F (N-Glycosidase F) is the most effective method of removing virtually all N-linked oligosaccharides from glycoproteins. PNGase F cleaves all asparagine-linked complex, hybrid, or high mannose oligosaccharides unless the core contains an  $\alpha(1\rightarrow3)$ -fucose. A tripeptide with the oligosaccharide-linked asparagine as the central residue is the minimal substrate for PNGase F (see **Figure 4**). The asparagine residue from which the glycan is removed is deaminated to aspartic acid (see **Figure 5**). The oligosaccharide is left intact and is suitable for further analysis.



**Figure 4.** Cleavage site and structural requirements for PNGase F.  
R<sub>1</sub> = N- and C-substitution by groups other than H  
R<sub>2</sub> = H or the rest of an oligosaccharide structure  
R<sub>3</sub> = H or  $\alpha(1\rightarrow6)$ fucose



**Figure 5.** Cleavage products from PNGase treatment of N-glycans.

Oligosaccharides containing a fucose  $\alpha(1\rightarrow3)$ -linked to the asparagine-linked N-acetylglucosamine (GlcNAc), commonly found in glycoproteins from plants or parasitic worms, are resistant to PNGase F. PNGase A (N-Glycosidase A), isolated from almond meal, must be used in this situation. However, PNGase A is ineffective when sialic acid is present on the N-linked oligosaccharide.

Other commonly used endoglycosidases such as Endoglycosidase H and the Endoglycosidase F series are not suitable for general deglycosylation of N-linked sugars because of their limited specificities and because they leave one N-acetylglucosamine residue attached to the asparagine.

Steric hindrance slows or inhibits the action of PNGase F on certain residues of glycoproteins. Denaturation of the glycoprotein by heating with SDS and 2-mercaptoethanol greatly increases the rate of deglycosylation.

Through sequential deglycosylation of monosaccharides using exoglycosidases, all complex oligosaccharides can be reduced to the trimannosyldiacetylchitobiose (Man<sub>3</sub>GlcNAc<sub>2</sub>) core. Complex N-linked glycans can be selectively hydrolyzed with a neuraminidase,  $\beta$ -galactosidase, and N-acetylglucosaminidase, available as part of the **Enzymatic Deglycosylation Kit (Cat. No. EDEGLY; page 83)**. Additional cleavage using fucosidases may be required in some situations.

## PNGase F

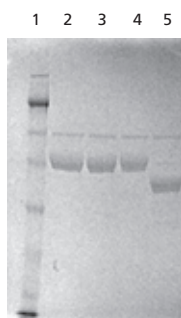
**Synonyms:** Glycopeptidase F; N-Glycosidase F; Peptide N-Glycosidase F; Peptide-N<sup>4</sup>-(acetyl-β-glucosaminy)-asparagine amidase  
EC 3.5.1.52  
Molecular mass: 36 kDa

PNGase F cleaves all asparagine-linked complex, hybrid, or high mannose oligosaccharides unless the core contains an α(1→3)-fucose. Detergent and heat denaturation increases the rate of cleavage up to 100 times. Most native proteins can still be completely N-deglycosylated, but incubation time must be increased. The optimal pH is 8.6 and the enzyme is active in the pH range of 6 to 10.

### Proteomics Grade PNGase F

Proteomics Grade PNGase F (**Cat. No. P7367**) is extensively purified and lyophilized from dilute potassium phosphate buffer to produce a stable product. The product is free from glycerol and other stabilizers that may interfere in sensitive glycoprotein analysis methods.

- Excellent for applications requiring N-linked deglycosylation (see **Figure 6**)
- Superior performance for on-blot, in-gel, and in solution digestion methods.
- High specific activity – ≥25,000 units/mg.
- Compatible for use in MALDI-TOF mass spectrometry.



**Figure 6.** SDS-PAGE analysis of native and PNGase F-treated α-1antitrypsin. The test sample (Lane 5) was deglycosylated in solution with 5 units of PNGase F for 1 hour at 37 °C prior to separation on SDS-PAGE. Note the shift in the mobility of the band upon deglycosylation.

#### Lanes

- 1: Molecular weight marker
- 2, 3, 4: Control, native α<sub>1</sub> antitrypsin
- 5: In-solution deglycosylated α<sub>1</sub> antitrypsin.

## PNGase F from *Elizabethkingia meningoseptica*

N-Glycosidase F; Peptide N-glycosidase; PNGase F from *Chryseobacterium meningosepticum*; PNGase F from *Flavobacterium meningosepticum* [83534-39-8] E.C. 3.5.1.52

Used to deglycosylate protein.

One unit will catalyze the release of N-linked oligosaccharides from 1 nanomole of denatured ribonuclease B in one minute at 37°C at pH 7.5 monitored by SDS-PAGE. One Sigma unit of PNGase F activity is equal to 1 IUB milliunit.

### ► Proteomics Grade, ≥95% (SDS-PAGE)

Suitable for both proteomics and glycobiology use; compatible with MALDI-TOF MS analysis

mol wt ~36 kDa

ship: wet ice store at: 2-8°C

P7367-50UN	50 units
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P7367-300UN	300 units
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### ► buffered aqueous solution

Solution in 20 mM Tris HCl, pH 7.5, 50 mM NaCl and 5 mM EDTA

ship: wet ice store at: 2-8°C

G5166-50UN	50 units
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G5166-100UN	100 units
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N-Glycanase

### ► recombinant, expressed in *Escherichia coli*

activity: ≥10 units/mg protein











Each set includes enzyme, two formulations of 5x reaction buffer (for routine and mass spectrometry downstream analysis), detergent and denaturation solutions

N-Glycanase is a registered trademark of Genzyme Corporation, Boston, MA, USA. N-Glycanase is covered by US Patent No 5,238,821.

ship: wet ice store at: 2-8°C

P9120-1SET	1 set
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## Key to Monosaccharide Symbols

	
β-D-Glucose (Glc)	β-D-Xylose (Xyl)
	
β-D-Mannose (Man)	α-N-Acetylneuraminic acid; Sialic acid (NeuNAc)
	
β-D-Galactose (Gal)	β-D-Glucuronic acid (GlcA)
	
β-D-N-Acetylglucosamine (GlcNAc)	α-L-Iduronic acid (IdoA)
	
β-D-N-Acetylgalactosamine (GalNAc)	α-L-Fucose (Fuc)

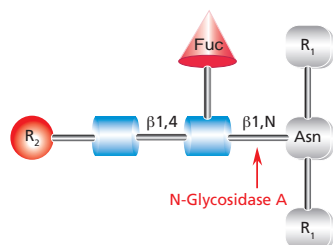
# Enzymatic Deglycosylation

## Glycopeptidase A

*Synonyms:* PNGase A; Peptide N-Glycosidase A  
EC 3.5.1.52

Molecular Mass: 52 kDa

Glycopeptidase A hydrolyzes oligosaccharides containing a fucose residue  $\alpha(1\rightarrow3)$ -linked to the asparagine-linked N-acetylglucosamine (see **Figure 7**). These types of glycans are resistant to PNGase F. Like PNGase F, the asparagine residue from which the glycan is removed is deaminated to aspartic acid. However, PNGase A is ineffective when sialic acid is present on the N-linked oligosaccharide.



**Figure 7.** Cleavage site and structural requirements for Glycopeptidase A (PNGase A).  
 $R_1$  = N- and C-substitution by groups other than H  
 $R_2$  = H or the rest of an oligosaccharide structure

## Native and Sequential N-Linked Glycan Strategies

For some glycoproteins, no cleavage by PNGase F occurs unless the protein is denatured. For others, some or all of the oligosaccharides can be removed from the native protein after extensive incubation (three days or longer) with PNGase F. PNGase F will remain active under reaction conditions for at least three days, making it suitable for extended incubations of native glycoproteins. Some particular residues, due to their location in the native protein structure, are resistant to PNGase F cleavage and cannot be removed unless the protein is denatured.

Endoglycosidases F1, F2, and F3 are less sensitive to protein conformation than PNGase F and are more suitable for deglycosylation of native proteins. Endoglycosidase F1 (Endo F1) cleaves asparagine-linked or free high mannose (oligomannose) and hybrid structures, while Endoglycosidase F2 (Endo F2) and Endoglycosidase F3 (Endo F3) have the ability to cleave complex structures. The linkage specificities of Endoglycosidases F1, F2, and F3 suggest a general strategy for deglycosylation of proteins that may remove all classes of N-linked oligosaccharides without denaturing the protein. As discussed previously, complex oligosaccharides can be reduced to the trimannosyldiacetylchitobiose ( $\text{Man}_3\text{GlcNAc}_2$ ) core using neuraminidase,  $\beta$ -galactosidase, N-acetylglucosaminidase, and fucosidases as required. The remaining trimannosyldiacetylchitobiose core structures can be removed with Endoglycosidase F3.

The **Native Protein Deglycosylation Kit (Cat. No. NDEGLY)**, see page 83) supplies all three of these enzymes (Endo F1, Endo F2 and Endo F3) with reaction buffers and detailed interactions.

## Glycopeptidase A from almonds

N-Glycosidase A; N-linked-glycopeptide-(N-acetyl- $\beta$ -D-glucosaminy)-L-asparagine amidohydrolase; PNGase A  
[83534-39-8] E.C. 3.5.1.52

### ► buffered aqueous glycerol solution, $\geq 0.05$ unit/mL

Solution in 50% glycerol containing 50 mM citrate-phosphate buffer, pH 5.0

One unit will hydrolyze 1.0  $\mu$ mole of ovalbumin glycopeptide per min at pH 5.0 at 37°C.

store at: -20°C

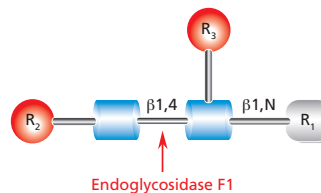
G0535-.005UN

0.005 unit

## Endoglycosidase F1

*Synonyms:* Endo F1; Endo- $\beta$ -N-acetylglucosaminidase F1

Endo F1 cleaves between the two N-acetylglucosamine residues in the N-linked diacetylchitobiose glycan core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine (see **Figure 8**).



**Figure 8.** Cleavage site and structural requirements for Endoglycosidase F1.

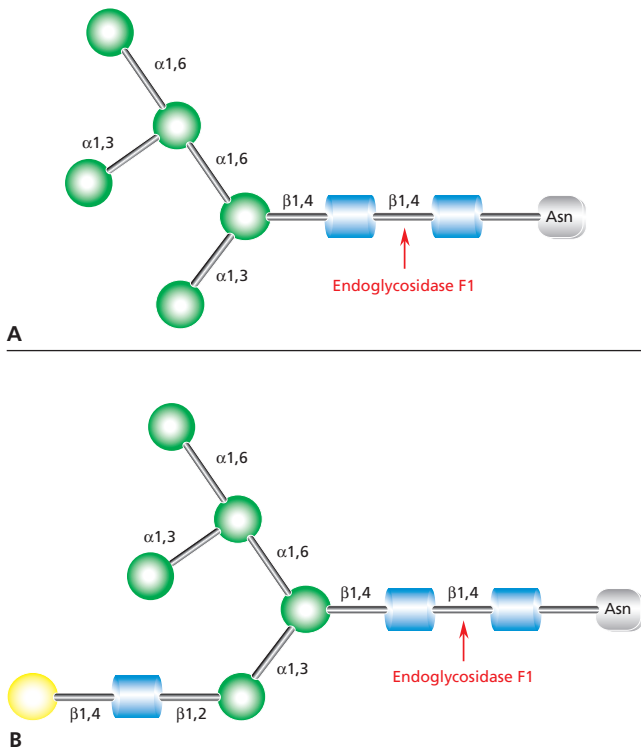
$R_1$  = H or Asn

$R_2$  = Oligomannose or hybrid configuration

$R_3$  = H or  $\alpha(1\rightarrow6)$ fucose

Endo F1 cleaves asparagine-linked or free high mannose (oligomannose) and hybrid structures, but not complex oligosaccharides (see **Figure 9**). Core fucosylation of hybrid structures reduces the rate of cleavage by Endo F1 more than 50-fold. Endo F1 will cleave sulfated high mannose oligosaccharides whereas Endoglycosidase H will not. Endo F1 may be used under native or non-denaturing deglycosylation conditions.

# Enzymatic Deglycosylation

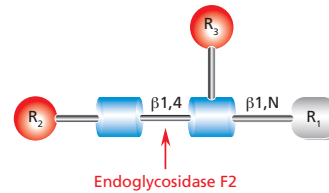


**Figure 9.** Cleavage site for Endoglycosidase F1 in **(A)** high mannose glycans and **(B)** hybrid glycans.

## Endoglycosidase F2

*Synonyms:* Endo F2; Endo- $\beta$ -N-acetylglucosaminidase F2

Endo F2 cleaves between the two N-acetylglucosamine residues in the N-linked diacetylchitobiose glycan core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine (see **Figure 10**).



**Figure 10.** Cleavage site and structural requirements for Endoglycosidase F2.











$R_1$  = H or Asn

$R_2$  = Biantennary or oligomannose configurations

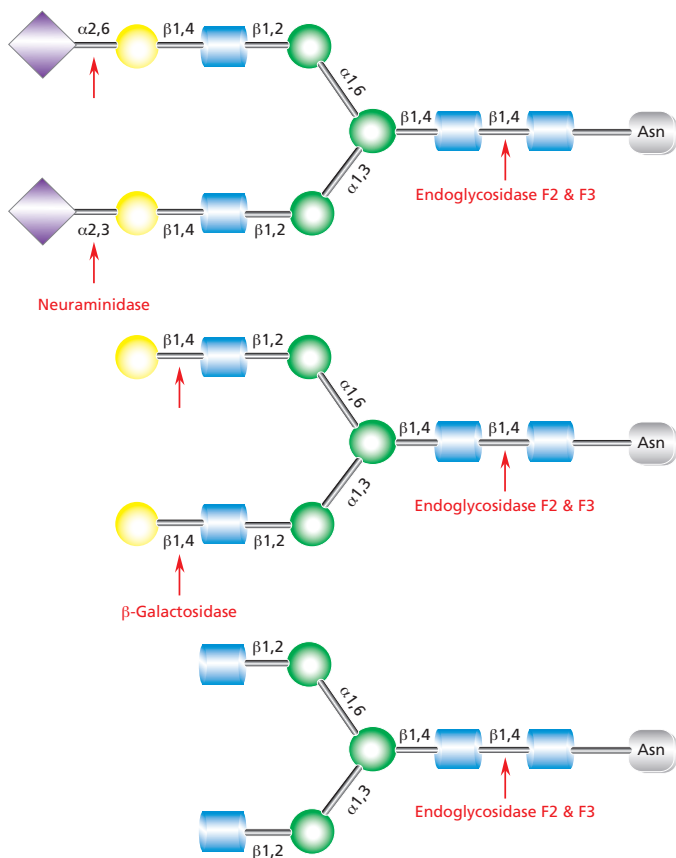
$R_3$  = H or  $\alpha(1\rightarrow6)$  fucose

Endo F2 cleaves biantennary complex oligosaccharides (see **Figure 11**). High mannose oligosaccharides are cleaved at a rate reduced 20-fold compared to that of complex structures. Endo F2 will not cleave hybrid structures. Fucosylation has little effect on Endo F2 cleavage of biantennary structures. Endo F2 is useful under native or non-denaturing deglycosylation conditions.

### Key to Monosaccharide Symbols

	
$\beta$ -D-Glucose (Glc)	$\beta$ -D-Xylose (Xyl)
	
$\beta$ -D-Mannose (Man)	$\alpha$ -N-Acetylneuraminic acid; Sialic acid (NeuNAc)
	
$\beta$ -D-Galactose (Gal)	$\beta$ -D-Glucuronic acid (GlcA)
	
$\beta$ -D-N-Acetylglucosamine (GlcNAc)	$\alpha$ -L-Iduronic acid (IdoA)
	
$\beta$ -D-N-Acetylgalactosamine (GalNAc)	$\alpha$ -L-Fucose (Fuc)

# Enzymatic Deglycosylation

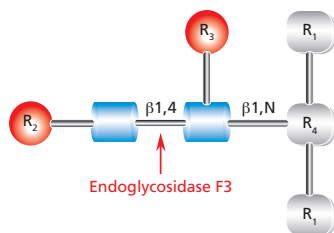


**Figure 11.** Cleavage site for Endoglycosidases F2 and F3 in a complex biantennary glycan. Sequential degradation using the exoglycosidic enzymes neuraminidase and  $\beta$ -galactosidase to remove terminal monosaccharides is shown.

## Endoglycosidase F3

*Synonyms:* Endo F3; Endo- $\beta$ -N-acetylglucosaminidase F3

Endo F3 cleaves between the two N-acetylglucosamine residues in the N-linked diacetylchitobiose glycan core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine (see **Figure 12**).



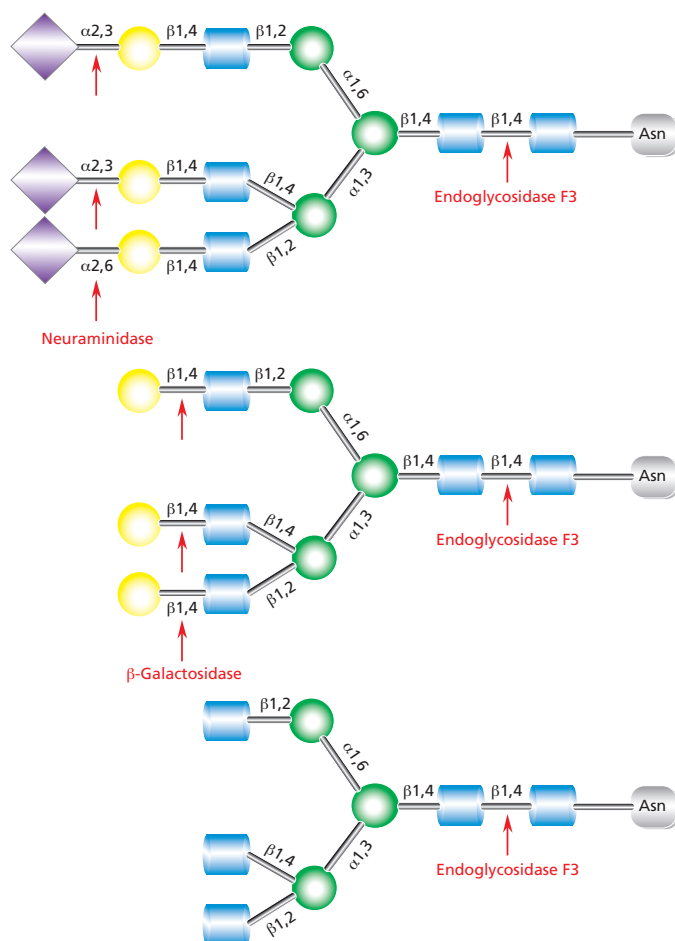
**Figure 12.** Cleavage site and structural requirements for Endoglycosidase F3.

- R<sub>1</sub> = N- and C-substitution by groups other than H
- R<sub>2</sub> = Biantennary or triantennary complex oligosaccharide or trimannosyl(Man<sub>3</sub>) core
- R<sub>3</sub> = H or  $\alpha(1\rightarrow6)$ fucose
- R<sub>4</sub> = Asn (Asn or H if fucosylated at R3)











Endo F3 has no activity on oligomannose and hybrid molecules. Endo F3 cleaves non-fucosylated biantennary and triantennary complex oligosaccharides at a slow rate, but only if peptide-linked (see **Figures 11 and 13**). Core fucosylation of biantennary structures increases activity up to 400-fold. Core fucosylated biantennary structures are efficient substrates for Endo F3, even as free oligosaccharides. Endo F3 will also cleave fucosylated trimannosyl core structures on free and protein-linked oligosaccharides.

Native deglycosylation of complex tetraantennary glycans requires sequential hydrolysis to the trimannosyldiacetylchitobiose (Man<sub>3</sub>GlcNAc<sub>2</sub>) core prior to cleavage using Endo F3 (see **Figure 14**).

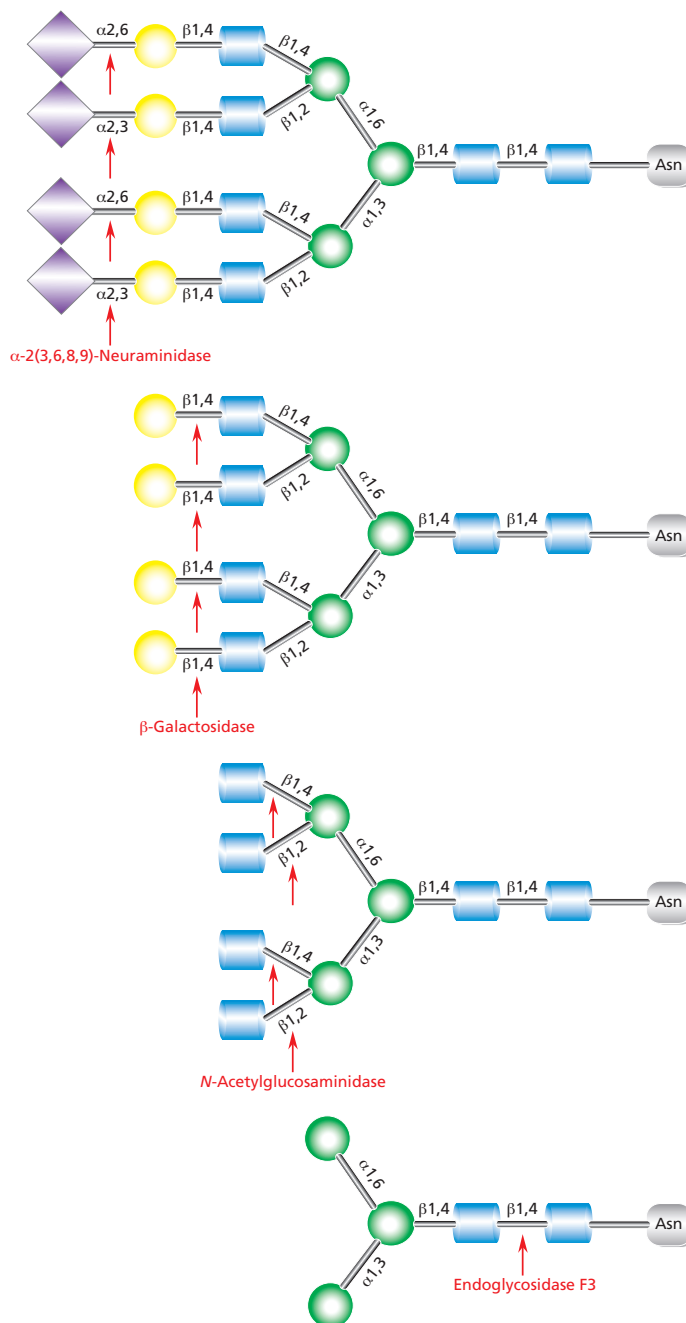
# Enzymatic Deglycosylation



**Figure 13.** Cleavage site for Endoglycosidases F3 in complex triantennary glycan. Sequential degradation using the exoglycosidic enzymes neuraminidase and  $\beta$ -galactosidase to remove terminal monosaccharides is shown.

Key to Monosaccharide Symbols	
	
$\beta$ -D-Glucose (Glc)	$\beta$ -D-Xylose (Xyl)
	
$\beta$ -D-Mannose (Man)	$\alpha$ -N-Acetylneuraminic acid; Sialic acid (NeuNAc)
	
$\beta$ -D-Galactose (Gal)	$\beta$ -D-Glucuronic acid (GlcA)
	
$\beta$ -D-N-Acetylglucosamine (GlcNAc)	$\alpha$ -L-Iduronic acid (IdoA)
	
$\beta$ -D-N-Acetylgalactosamine (GalNAc)	$\alpha$ -L-Fucose (Fuc)

# Enzymatic Deglycosylation



**Figure 14.** Sequential deglycosylation of complex tetraantennary glycan. The terminal monosaccharides are sequentially removed using the exoglycosidic enzymes neuraminidase,  $\beta$ -galactosidase, and N-acetylglucosaminidase, until the trimannosyl diacetylchitobiose core remains for subsequent cleavage by Endo F3.

# Enzymatic Deglycosylation

## Endoglycosidase H

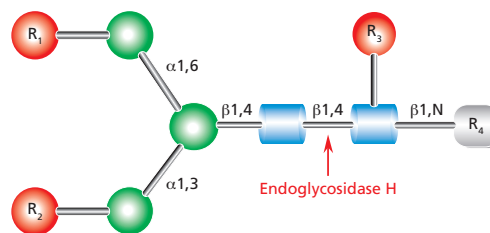
**Synonyms:**  $\beta$ -N-Acetylglucosaminidase H

Endoglycosidase H cleaves between the N-acetylglucosamine residues of the chitobiose core of N-linked glycans, leaving one N-acetylglucosamine residue attached to the asparagine (see **Figure 15**). The specificity of this enzyme is such that oligomannose and most hybrid types of glycans, including those that have a fucose residue attached to the core structure, are cleaved whereas complex type glycans are not released. Thus, this enzyme is extremely useful for selective release of high mannose (oligomannose) or hybrid type glycans from glycoproteins. The enzyme is also active against dolichol-linked glycans containing these structures.

The functional pH range for Endoglycosidase H is 5.0 to 6.0, with the optimum pH at 5.5. No loss of activity was observed during incubation at 37 °C for 48 hours over the pH range 4.5 to 8.5. However, below pH 4.5, activity is rapidly lost.

## Endoglycosidases

Name	Unit Definition	Form	Packaging	Cat. No.
Endoglycosidase F1 from <i>Chryseobacterium meningosepticum</i>	One unit will release N-linked oligosaccharides from 1 $\mu$ mole of denatured Ribonuclease B in 1 minute at 37 °C, pH 5.5.	Aseptically filled solution in 20 mM Tris-HCl pH 7.5	Supplied with 5x Reaction Buffer, 250 mM NaH <sub>2</sub> PO <sub>4</sub> , pH 5.5.	E9762-1UN
Endoglycosidase F2 from <i>Chryseobacterium meningosepticum</i>	One unit will release N-linked oligosaccharides from 1 $\mu$ mole of denatured porcine fibrinogen in 1 minute at 37 °C, pH 4.5.	Aseptically filled solution in 10 mM sodium acetate, 25 mM sodium chloride, pH 4.5	Supplied with 5x Reaction Buffer, 250 mM sodium acetate, pH 4.5	E0639-.2UN
Endoglycosidase F3 from <i>Elizabethkingia (Chryseobacterium/Flavobacterium) meningosepticum</i>	One unit will release N-linked oligosaccharides from 1 $\mu$ mole of denatured porcine fibrinogen in 1 minute at 37 °C, pH 4.5.	Aseptically filled solution in 20 mM Tris-HCl, pH 7.5	Supplied with 5x Reaction Buffer, 250 mM sodium acetate, pH 4.5	E2264-.2UN
Endoglycosidase H from <i>Streptomyces griseus</i>	One unit will hydrolyze 1.0 $\mu$ mole of N-acetyl-( <sup>14</sup> C)Asn(GlcNAc) <sub>2</sub> (Man) <sub>5</sub> per min at pH 5.0 at 37 °C.	Lyophilized from a solution containing 10 mM Tris HCl, pH 7.2. This product does not contain a protein stabilizer and must be reconstituted with a solution containing BSA or another stabilizer.	-	E2406-.1UN
Endoglycosidase H from <i>Streptomyces plicatus</i> expressed in <i>Escherichia coli</i>	One unit will hydrolyze 1.0 $\mu$ mole of dansyl-Asn-(GlcNAc) <sub>2</sub> (Man) <sub>5</sub> per min at pH 5.5 at 37 °C.	Solution in 0.05 M sodium phosphate, pH 7, containing 25 mM EDTA and preservative	-	E7642-1UN
Endoglycosidase H from <i>Streptomyces plicatus</i> expressed in <i>Escherichia coli</i>	One unit will release N-linked oligosaccharides from 60 $\mu$ moles of ribonuclease B per hr at 37 °C at pH 5.5.	Solution in 20 mM Tris HCl, pH 7.5, 25 mM NaCl	-	A0810-1UN
Endo- $\beta$ -galactosidase expressed in <i>Escherichia coli</i>	One unit will release 1.0 $\mu$ mole of reducing sugar from bovine corneal keratan sulfate per minute at 37 °C, pH 5.8.	Aseptically filled solution in 20 mM Tris-HCl, pH 7.5	-	G6920-.5UN
Lacto-N-biosidase from <i>Streptococcus</i> sp.	One unit will hydrolyze 1 $\mu$ mol of pyridylamino lacto-N-tetraose per minute at 37 °C at pH 5.5.	Solution in 50 mM sodium acetate buffer, pH 5.5, containing 0.05% Brij® 58.	-	L9903-1VL



**Figure 15.** Cleavage site and structural requirements for Endoglycosidase H.  
 R<sub>1</sub> = Oligomannose (2-150 mannose residues)  
 R<sub>2</sub> = H-, mono- or oligosaccharide at the C-2 or C-4 position  
 R<sub>3</sub> = H or  $\alpha$ (1 $\rightarrow$ 6)fucose  
 R<sub>4</sub> = Asn or dolichol pyrophosphate

### Key to Monosaccharide Symbols

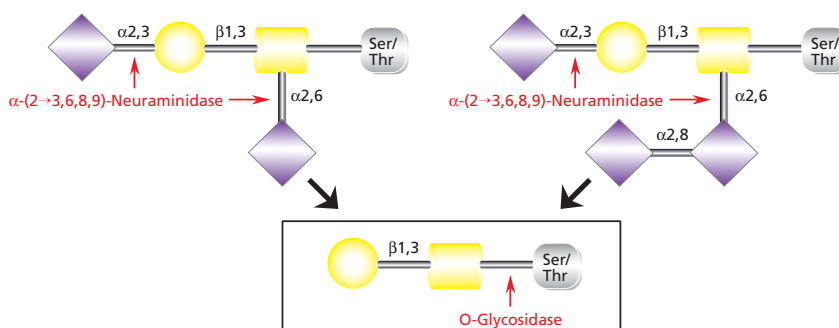
	$\beta$ -D-Glucose (Glc)		$\beta$ -D-Xylose (Xyl)
	$\beta$ -D-Mannose (Man)		$\alpha$ -N-Acetylneuraminic acid; Sialic acid (NeuNAc)
	$\beta$ -D-Galactose (Gal)		$\beta$ -D-Glucuronic acid (GlcA)
	$\beta$ -D-N-Acetylglucosamine (GlcNAc)		$\alpha$ -L-Iduronic acid (IdoA)
	$\beta$ -D-N-Acetylgalactosamine (GalNAc)		$\alpha$ -L-Fucose (Fuc)

# Enzymatic Deglycosylation

## O-Linked Glycan Strategies

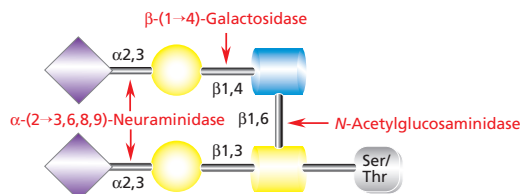
While most N-linked oligosaccharides can be removed using PNGase F, a comparable enzyme for removing intact O-linked sugars has not been identified. Monosaccharides must be sequentially hydrolyzed by a series of exoglycosidases until only the Gal- $\beta$ (1 $\rightarrow$ 3)-GalNAc core remains. At that point, O-glycosidase can remove the intact core structure with no modification of the serine or threonine residue. Denaturation of the glycoprotein does not appear to significantly enhance O-deglycosylation.

Any modification of the core structure can block the action of O-glycosidase. The most common modifications of the core Gal- $\beta$ (1 $\rightarrow$ 3)-GalNAc are mono-, di-, and trisialylation. These residues are easily removed by  $\alpha$ -(2 $\rightarrow$ 3,6,8,9)-neuraminidase. Only this enzyme is capable of efficient cleavage of the NeuAc- $\alpha$ (2 $\rightarrow$ 8)-NeuAc bond (see **Figure 16**). Another commonly occurring O-linked hexasaccharide structure contains  $\beta$ (1 $\rightarrow$ 4)-linked galactose (Gal) and  $\beta$ (1 $\rightarrow$ 6)-linked N-acetylglucosamine (GlcNAc) as well as sialic acid. Hydrolysis of this glycan requires, in addition to neuraminidase, a  $\beta$ (1 $\rightarrow$ 4)-specific galactosidase and an N-acetylglucosaminidase. A galactosidase that is not linkage specific will hydrolyze  $\beta$ (1 $\rightarrow$ 3)-galactose from the core glycan, leaving an O-linked GalNAc residue that cannot be removed by O-glycosidase.



**Figure 16.** In sequential glycolytic cleavage, disialylated and trisialylated O-linked glycans have the sialic acid residues (NeuNAc) removed by  $\alpha$ (2 $\rightarrow$ 3,6,8,9) neuraminidase. The Core 1 type glycan is then cleaved from the O-linkage by O-glycosidase.

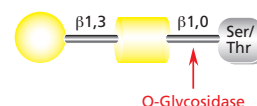
$\beta$ (1 $\rightarrow$ 4)-Galactosidase and  $\beta$ -N-acetylglucosaminidase can be used for the hydrolysis of these and any other O-linked structures containing  $\beta$ (1 $\rightarrow$ 4)-linked galactose (Gal) or  $\beta$ -linked N-acetylglucosamine (GlcNAc) such as polylactosamine (see **Figure 17**). Less common modifications that have been found on O-linked oligosaccharides include  $\alpha$ -linked galactose (Gal) and  $\alpha$ -linked fucose. N-Acetylglucosamine attached directly to the peptide backbone (found on nuclear proteins) and  $\alpha$ -linked N-acetylglucosamine (found in mucins) have also been reported. Additional exoglycosidases are necessary for complete O-deglycosylation when these residues are present. Fucose and mannose directly O-linked to proteins cannot presently be removed enzymatically.



**Figure 17.** Disialylated O-linked Core 2 hexasaccharide is sequentially degraded by (1) removal of sialic acid residues (NeuNAc) using  $\alpha$ (2 $\rightarrow$ 3,6,8,9) neuraminidase, (2) removal of  $\beta$ (1 $\rightarrow$ 4)-galactose (Gal) residues using  $\beta$ (1 $\rightarrow$ 4)-galactosidase, and (3) removal of N-acetylglucosamine (GlcNAc) using N-acetylglucosaminidase. The remaining Core 1 type glycan can then be cleaved from O-linkage using O-glycosidase as shown in Figure 16.

### O-Glycosidase

**Synonyms:** Endo- $\alpha$ -N-acetylgalactosaminidase; O-Glycanase  
O-Glycosidase hydrolyzes the serine or threonine-linked unsubstituted O-glycan core [Gal- $\beta$ (1 $\rightarrow$ 3)-GalNAc] (see **Figure 18**). Any modification of the core structure can block the action of O-glycosidase.



**Figure 18.** Cleavage site and structural requirements for O-Glycosidase. Any modification of the core structure will prevent cleavage.

### O-Glycosidase from *Streptococcus pneumoniae*

Endo- $\alpha$ -N-acetylgalactosaminidase; O-Glycanase  
[9032-92-2] E.C. 3.2.1.97

#### ► recombinant, expressed in *Escherichia coli*, buffered aqueous solution

Solution in 50 mM sodium phosphate, pH 7.5  
Supplied with 5 $\times$  Reaction Buffer, 250 mM NaH<sub>2</sub>PO<sub>4</sub> pH 5.0.

One unit will hydrolyze 1  $\mu$ mole of *p*-nitrophenyl galacto-N-bioside ( $\beta$ -Gal-(1 $\rightarrow$ 3)- $\alpha$ -GalNAc-1 $\rightarrow$ OC<sub>6</sub>H<sub>4</sub>NO<sub>2</sub>) per min at 37  $^{\circ}$ C at pH 6.5.

Screened for presence of:  $\beta$ -galactosidase,  $\alpha$ -mannosidase,  $\beta$ -hexosaminidase,  $\alpha$ -fucosidase, neuraminidase, and proteases. See Certificate of Analysis for lot specific information.

store at: 2-8 $^{\circ}$ C

G1163-.04UN

0.04 unit

## Deglycosylation Kits

### GlycoProfile™ I Enzymatic In-gel N-Deglycosylation Kit

Glycosylation often leads to problems in subsequent protein analysis procedures. Glycopeptides generally do not readily ionize during MS analysis leading to insufficient spectral data. Furthermore, proteolytic digestion of the native glycoprotein is often incomplete due to steric hindrance by the oligosaccharides. Removal of the carbohydrate groups from a glycoprotein prior to protein identification is preferred.

Sigma's GlycoProfile I Enzymatic In-gel N-Deglycosylation Kit is optimized to provide a convenient and reproducible method to N-deglycosylate and typically digest protein samples in 1D or 2D polyacrylamide gel slices for subsequent MS or HPLC analysis. The procedure is suitable for Coomassie® Blue and Colloidal Coomassie stained gels and may be used with gels silver stained and destained using Sigma's ProteoSilver™ Plus Kit (Cat. No. PROTSIL2). GlycoProfile Enzymatic In-gel N-Deglycosylation Kit includes the enzymes and reagents necessary for N-linked deglycosylation and tryptic digestion. The samples can then be desalted and concentrated for analysis by MALDI-TOF or electrospray MS.

#### Features and Benefits

- Provides all components for in-gel deglycosylation and trypsinization of protein samples—conveniently prepares deglycosylated protein samples for analysis by MS or HPLC
- Utilizes PNGase F for the enzymatic removal of N-linked glycans—proteins remain intact, unlike the use of chemical deglycosylation which can degrade the protein
- Includes Proteomics Grade PNGase F and Trypsin—highly purified enzymes possess no unwanted activities or additives to complicate analysis
- PNGase F is supplied lyophilized from a low salt buffer—allows reconstitution of the enzyme to any concentration needed
- Works in solution or with gel slices—allows choice of methods



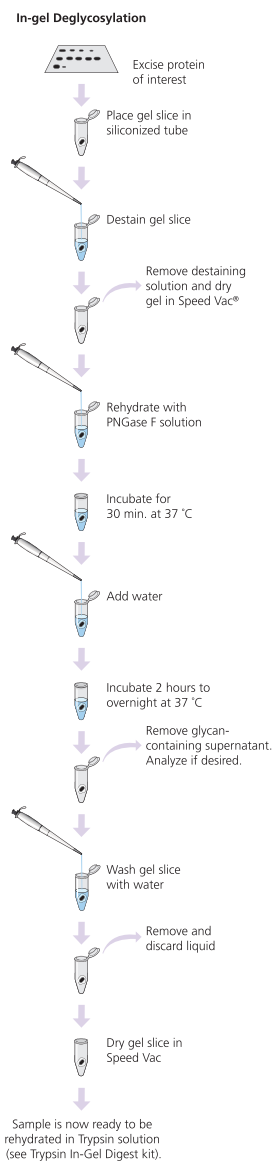
**Figure 19.** Components of GlycoProfile I, In-Gel Deglycosylation Kit (Cat. No. PP0200).

### GlycoProfile™ I, In-Gel Deglycosylation Kit

Each kit contains reagents sufficient to deglycosylate and digest up to 10 samples.

#### Components

PNGase F *Elizabethkingia meningoseptica* (Sigma P7367) 50 units  
 Trypsin porcine pancreas (Sial T6567) 20 µg  
 Destaining Solution  
 Trypsin Solubilization Reagent  
 Trypsin Reaction Buffer  
 Invertase Glycoprotein Standard (Sigma I0408)  
 Acetonitrile (Aldrich 494445) 50 ml  
 Peptide Extraction Solution  
 store at: 2-8°C



PP0200-1KT

1 kit

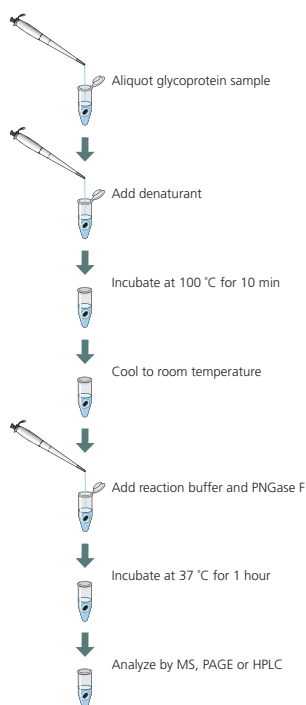
# Enzymatic Deglycosylation

## GlycoProfile™ II Enzymatic In-solution N-Deglycosylation Kit

The GlycoProfile Enzymatic In-solution N-Deglycosylation Kit has been optimized to provide a convenient and reproducible method to remove N-linked glycans from glycoproteins and is compatible with subsequent MALDI-TOF mass spectrometric analysis without interference from any of the reaction components. The kit contains sufficient enzyme, glycoprotein standard, and reagents, for a minimum of 20 reactions when the sample size is between one to two mg of a typical glycoprotein.

### Features and Benefits

- Provides all components for in-solution N-linked deglycosylation of protein samples—*conveniently prepares deglycosylated protein samples for analysis by MS, HPLC, and PAGE*
- Reagents are optimized for direct MS analysis—*no need for post-reaction sample clean up*
- Utilizes PNGase F for the enzymatic removal of N-linked glycans—*proteins remain intact, unlike the use of chemical deglycosylation which can degrade the protein*
- Includes Proteomics Grade PNGase F and Trypsin—*highly purified enzymes possess no unwanted activities or additives to complicate analysis*
- PNGase F is supplied lyophilized from a low salt buffer—*allows reconstitution of the enzyme to any concentration needed*



In-solution Deglycosylation

## GlycoProfile™ II, Enzymatic In-Solution N-Deglycosylation Kit

Contains sufficient reagents for a minimum of 20 reactions when the sample size is between one to two mg of a typical glycoprotein.

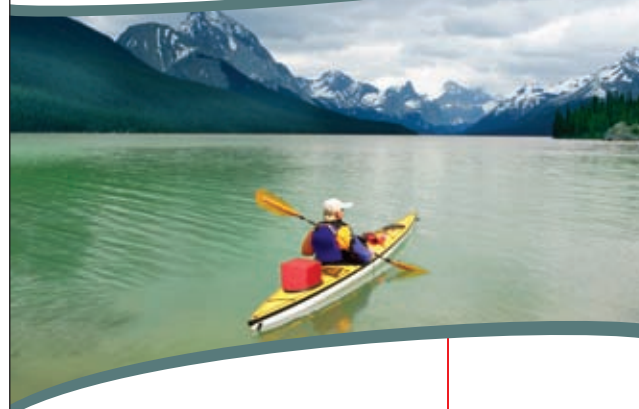
### Components

PNGase F Elizabethkingia meningoseptica (Sigma P7367) 50 units  
Ribonuclease B from bovine pancreas (Sigma R7884) 0.5 mg  
10× Reaction Buffer 1 vial  
Octyl β-D-glucopyranoside (Sial O9882) 100 mg  
2-Mercaptoethanol (Sigma M3148) 0.90 ml  
store at: 2-8°C

PP0201-1KT

1 kit

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## Enzymatic Protein Deglycosylation Kit

The EDEGLY Kit contains the enzymes and reagents required for deglycosylation of both N and O-linked glycans.

### Features and Benefits

- Single reaction at neutral pH—*no need to adjust conditions for each glycosidase reaction*
- Native & denaturing procedures—*allows flexibility to ensure resulting deglycosylated proteins are compatible with down-stream analysis*
- Uses highly purified glycosidases, free of contaminating proteases—*no degradation of protein results in clear, reliable results*
- Includes fetuin which has sialylated N and O-linked oligosaccharides—*provides a control to check for efficiency of the deglycosylation*
- Each reaction deglycosylates 200 µg of glycoprotein—*yields enough deglycosylated protein for further analysis*
- Contains sufficient reagent for the deglycosylation of ~2 mg of protein—*allows for the processing of multiple samples*

## Enzymatic Protein Deglycosylation Kit

### Components

PNGase F 1 vial  
 O-Glycosidase 20 µl  
 α-2(3,6,8,9)-Neuraminidase 20 µl  
 Fetuin Control 0.5 mg  
 5× Reaction Buffer 0.2 ml  
 Denaturation Solution 0.1 ml  
 Triton® X-100 0.1 ml  
 β-1→4-Galactosidase, positionally specific 20 µl  
 β-N-Acetylglucosaminidase 20 µl  
 ship: wet ice store at: 2-8°C

EDEGLY-1KT 1 kit

## Native Protein Deglycosylation Kit

The NDEGLY Kit is intended for the deglycosylation of N-linked oligosaccharides from glycoproteins under native conditions. Particular residues, due to their location in the native protein may be resistant to the traditional deglycosylation methods using PNGase F. The NDEGLY Kit utilizes endoglycosidases which are less sensitive to protein conformation than PNGase F and, therefore, more suitable for deglycosylation of native proteins.

### Features and Benefits

- Enzymes included are less sensitive to protein conformation than PNGase F—*allows the deglycosylation of native proteins*
- Uses highly purified glycosidases, free of contaminating proteases—*no degradation of protein provides reliable, clear data*
- Each reaction deglycosylates 200 µg of glycoprotein—*yields enough deglycosylated protein for further analysis*
- Contains sufficient reagent for the deglycosylation of ~2 mg of protein—*allows for the processing of multiple samples*


## Native Protein Deglycosylation Kit


### Components

Endoglycosidase F1 0.3 un  
 Endoglycosidase F2 0.1 un  
 Endoglycosidase F3 0.1 un  
 Endoglycosidase F1 reaction buffer 200 µl  
 Endoglycosidase F2 & 3 reaction buffer 200 µl  
 ship: wet ice store at: 2-8°C

NDEGLY-1KT 1 kit

## The Enzyme Explorer





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- Cofactors

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