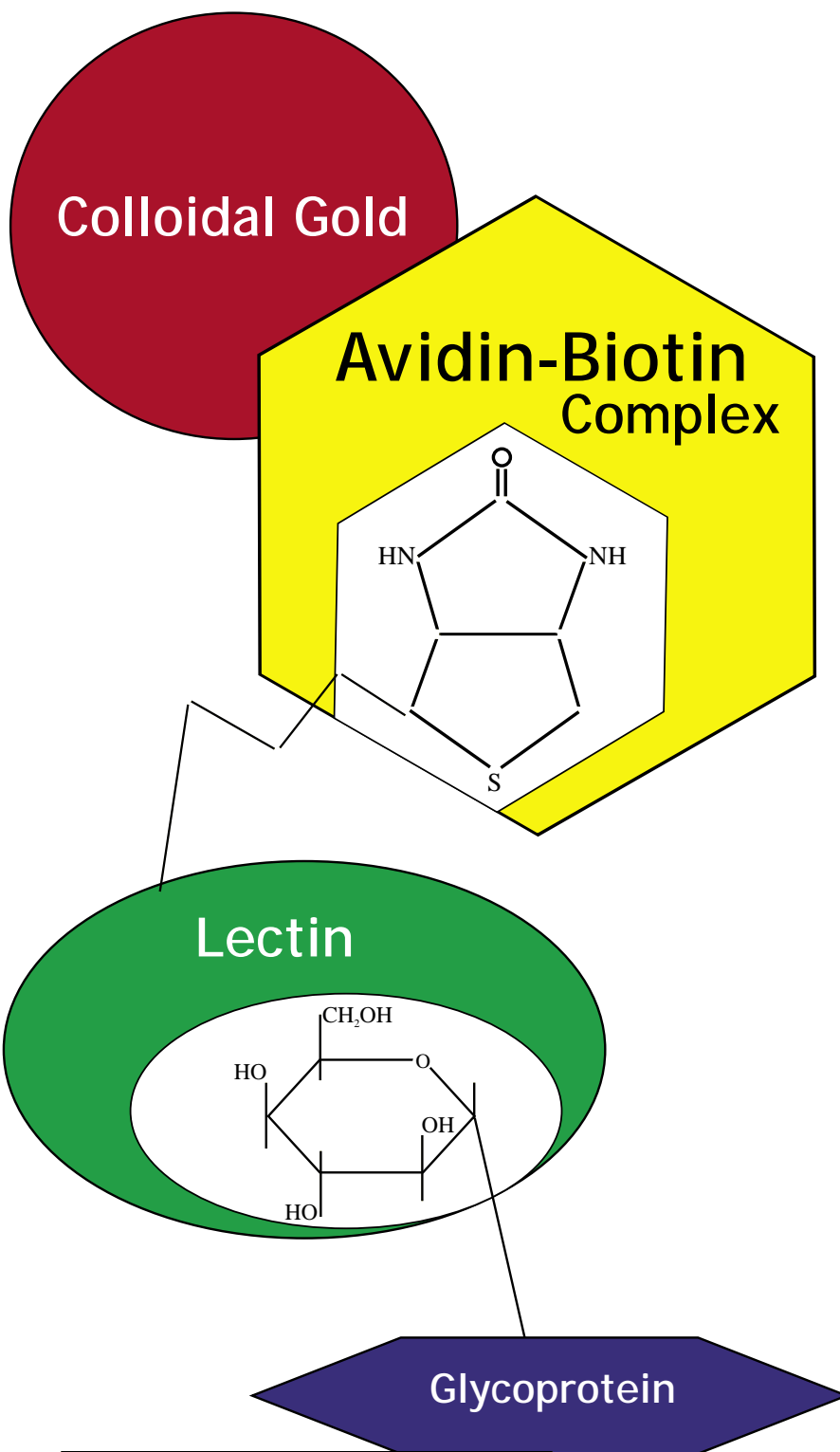


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Table of Contents

Alphabetical Listing of Lectins by Latin Name

<u>Lectin (latin name)</u>	<u>Common Name</u>	<u>Abbreviation</u>	<u>Page Number</u>
<i>Allium sativum</i>	garlic	ASA	2
<i>Amaranthus caudatus</i>	summer poinsettia	ACA	3
<i>Arum maculatum</i>	lords and ladies tubers	AMA	4
<i>Dioclea grandiflora</i>	legume	DGL	5
<i>Glechoma hederacea</i>	ground ivy	GHA	6
<i>Hippeastrum hybrid</i>	amaryllis	HHA	7
<i>Iris hybrid</i>	Dutch Iris	IRA	8
<i>Marasmiium oreades</i>	mushroom	MOA	9
<i>Morniga</i>	black mulberry tree	MNA-G, MNA-M	10
<i>Polygonatum multiflorum</i>	Solomon's seal	PMA	11
<i>Polyporus Squamosus</i>	mushroom	PSL	12

Alphabetical Listing of Lectins by Abbreviation

<u>Abbreviation</u>	<u>Lectin</u>	<u>Page No.</u>
ACA	<i>Amaranthus caudatus</i>	3
AMA	<i>Arum maculatum</i>	4
ASA	<i>Allium sativum</i>	2
DGL	<i>Dioclea grandiflora</i>	5
GHA	<i>Glechoma hederacea</i>	6
HHA	<i>Hippeastrum hybrid</i>	7
IRA	<i>Iris hybrid</i>	8
MOA	<i>Marasmiium oreades</i>	9
MNA-G, MNA-M	<i>Morniga</i>	10
PMA	<i>Polygonatum multiflorum</i>	11
PSL	<i>Polyporus Squamosus</i>	12

Numerical Listing of Lectin

(by catalog number of crude or pure form)

<u>Catalog No.</u>	<u>Lectin</u>	<u>Page Number</u>
L-8007	<i>Allium sativum</i>	ASA
L-8008	<i>Hippeastrum hybrid</i>	HHA
L-8009	<i>Polygonatum multiflorum</i>	PMA
L-8010	<i>Iris hybrid</i>	IRA
L-8012	<i>Arum maculatum</i>	AMA
L-8201	<i>Amaranthus caudatus</i>	ACA
L-9001	<i>Marasmiium oreades</i>	MOA
L-9002	<i>Dioclea grandiflora</i>	DGL
L-9003	<i>Glechoma hederacea</i>	GHA
L-9004	<i>Morniga</i>	MNA-M
L-9005	<i>Morniga</i>	MNA-G
L-9006	<i>Polyporus Squamosus</i>	PSL

Allium sativum ASA (garlic)

Two new mannose-binding lectins were isolated from garlic (*Allium sativum*, ASA) and ramsons (*Allium ursinum*, AUA) bulbs, of the family Alliaceae. ASA reacted strongly with a synthetic linear (1---3)-alpha-D-mannan and *S. cerevisiae* mannan, weakly with a synthetic (1---6)-alpha-D-mannan, and failed to precipitate with galactomannans from *T. gropengiesseri* and *T. lactis-condensi*, a linear mannopentaose, and murine IgM. On the other hand, AUA gave a strong reaction of precipitation with murine IgM, and good reactions with *S. cerevisiae* mannan and both synthetic linear mannans, suggesting that the two lectins have somewhat different binding specificities for alpha-D-mannosyl units. These two lectins exhibit different binding specificity for alpha-D-mannosyl units.(1)

Lectin cDNA clones for two different lectins from garlic (*Allium sativum* L.) bulbs, ASAI and ASAIL (ASA, *Allium sativum* agglutinin), were isolated and characterized. The first lectin, ASAI, is a heterodimer composed of two different subunits of 11.5 kDa and 12.5 kDa. It is translated from an mRNA of 1400 nucleotides encoding a polypeptide of 306 amino acids with two very similar domains. N-terminal sequencing of the two polypeptides of the mature lectin confirmed that both subunits are derived from the same precursor and that each corresponds to one of the two domains in the sequence. In contrast to ASAI, the second garlic lectin, ASAIL, is a homodimer of two identical 12-kDa subunits. It is translated from an mRNA of approximately 800 nucleotides encoding a polypeptide of 154 amino acids. Interestingly, the coding region of the ASAIL cDNA clones is almost identical to that of the second domain of the ASAI cDNA clones.(2)

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1. Kaku H, et al. Carbohydr Res. 1992 May 22;229(2):347-53.
2. van Damme EJ, et al. Eur J Biochem. 1992 Jun 1;206(2):413-20.

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	N/A
Activity:	Agglutinates rabbit but not human erythrocytes.
Inhibitory Carbohydrate:	α (1,3)-linked mannosyl units.
Molecular Weight:	ASAI is a heterodimer composed of two different subunits of 11.5 kDa and 12.5 kDa. ASAIL is a homodimer of two identical 12-kDa subunits

Ordering Information

Description	Cat. No.	Size
ASA affinity purified	L-8007-1	1 mg
ASA FITC	F-8007-1	1 mg
ASA TRITC	R-8007-1	1 mg
ASA Texas Red®	T-8007-1	1 mg
ASA Biotin	BA-8007-1	1 mg
ASA Horseradish Peroxidase	H-8007-1	1 mg
ASA Alkaline Phosphatase	LA-8007-1	1 mg
ASA Ferritin	I-8007-1	1 mg
ASA Colloidal Gold	GP-8007	1 ml
ASA Immobilized	A-8007-1	1 ml

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***Amaranthus caudatus* ACA (Tassel flower, Inca wheat)**

A lectin, from *Amaranthus caudatus* L. has been isolated and purified from its seed. This lectin, Amaranthin, is a homodimer (62 kDa) composed of lectin polypeptides of 33-36 kDa, which are not held together by disulphide bonds (1,2). The protein is not glycosylated. Amaranthin readily forms a precipitate with glycoproteins and glycoconjugates containing Gal β (1,3) GalNAc α -units. Hapten inhibition of precipitate formation between amaranthin and a sialo-ovine submaxillary indicated that the T-antigen disaccharide and its α -linked glycosides (Gal β (1,3) GalNAc α -O-R; R=OH, methyl, -(CH₂)₈-COOH, allyl, o-nitrophenyl or benzyl) were the best inhibitors. N-acetylgalactosamine, the only monosaccharide which inhibited precipitation was 350-fold less effective than Gal β (1,3) GalNAc α -O-R. Sequence analysis revealed that the protein is N-terminally blocked (1). The amino acid composition of the lectin is typified by high concentrations of acidic and hydroxyamino acids and relatively large amounts of lysine, methionin and tryptophan for a plant protein (1).

Another report describes the purification of the Amaranthin by affinity chromatography on asialofetuin-linked amino activated silica (3). In contrast to the report by Rinderle et al. (2) Singh et al. (3) claim that *Amaranthus* lectin is a glycoprotein containing 1.65 % carbohydrate.

The *Amaranthus* lectin (ACA) agglutinates human ABO and sheep, goat, rat, rabbit and guinea-pig erythrocytes (3).

ACA may be a highly specific tool probe for the T- and cryptic T-antigens (1). ACA may be used with other lectins of close carbohydrate binding specificity to compliment the Gal β (1,3) GalNAc - linkage of the disaccharides. ACA is used as a histochemical probe for proliferating cells in sections of human colonic tissues (4).

ACA represents about 3.7% of the PBS - extracted protein in seed meal (1). This lectin is present in seeds only and not in stems or in leaves (2). A lectin of similar T-antigen-binding specificity is present in *Artocarpus integrifolia* seeds (AIA). In addition to ACA in the *A. caudatus* seeds, there are also two small chitin-binding proteins present. These proteins, called Ac-AMP1 and Ac-AMP2 have antimicrobial activity and show sequence similarity to hevein (5).

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1. Rinderle, S.J. et al., J. of Biological Chemistry 264, (1989) 16123-16131.
2. Rinderle, S.J. et al., Biochemistry 29 (1990) 10555-10561.
3. Singh J., et al., Plant Science 94 (1993) 47-53.
4. Boland, C. R., et al., Cancer Research 51, (1991) 657-665.
5. Broekaert, W.F., et al., Biochemistry 31, (1992) 4308-4313.

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	A,B,O
Activity:	ACA agglutinates human ABO and sheep, goat, rat, rabbit and guinea-pig erythrocytes (3).
Inhibitory Carbohydrate:	GalNAc (T-antigen disaccharide and its α -linked glycosides (GAL β (1,3) GalNAc α -O-R))
Molecular Weight:	This lectin, Amaranthin, is a homodimer (62 kDa) composed of lectin polypeptides of 33-36 kDa, which are not held together by disulphide bonds (1,2).

Ordering Information

<u>Description</u>	<u>Cat. No.</u>	<u>Size</u>
ACA crude	L-8201-100	100mg
ACA affinity purified	L-8201-1	1mg
	L-8201-5	5mg
ACA FITC	F-8201-1	1mg
ACA TRITC	R-8201-1	1mg
ACA Texas Red®	T-8201-1	1mg
ACA Biotin	BA-8201-1	1mg
ACA Horseradish Peroxidase	H-8201-1	1mg
ACA Alkaline Phosphatase	LA-8201-1	1mg
ACA Ferritin	I-8201-1	1mg
ACA Colloidal Gold	GP-8201-1	1ml
ACA Immobilized	A-8201-2	2ml

***Arum maculatum* AMA (lords and ladies tubers)**

A lectin was purified from the tubers of *Arum maculatum* (family Araceae). AMA is not a glycoprotein and has a subunit molecular weight of 14,600. It is specifically inhibited by N-acetyllactosamine (Gal beta 1,4GlcNAc), but asialoglycoproteins which contain N-acetyllactosamine structures are even more effective inhibitors of the lectin. (1) The recombinant lectin is composed of two different 12-kD lectin subunits.(2)

AMA is a non-glycosylated protein with 20-kDa molecular mass agglutinating human ejaculated spermatozoa, but not human erythrocytes. The agglutination was blocked in the presence of N-acetylneuraminic acid indicating that the lectin is sialoglycoprotein specific. *Chlamydia pneumoniae* strain AR-39 showed considerable potential to grow in murine L-929 fibroblast cells. Pretreatment of the cell monolayers with purified lectin reduced the entry and intracellular replication of *C. pneumoniae*. These results suggest that the isolated lectin prevents attachment by binding to a *C. pneumoniae* specific sialoglycoprotein receptor expressed on the surface of L-929 fibroblast cells.(3)

Arum maculatum extracts were tested in vitro for antimicrobial activity against *Staphylococcus aureus* ATCC 65538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi*, *Shigella flexneri*, *Proteus mirabilis* and *Candida albicans* ATCC 10231, using microbroth dilution technique according to National Committee for Clinical Laboratory Standards (NCCLS). This research showed that *Arum maculatum*, *Datura stramonium*, *Geranium asphodeloides* and *Equisetum telmateia* petroleum ether extracts had MIC values of 39.1µg/ml, 78.1µg/ml, 78.1µg/ml and 39.1µg/ml, respectively against *Staphylococcus epidermidis*.(4)

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2. Van Damme EJ, et al. *Plant Physiol*. 1995 Apr;107(4):1147-58.
3. Mladenov IV et. al. *FEMS Immunol Med Microbiol*. 2002 Feb 18;32(3):249-54.
4. Uzun E, Sariyar G, et al. *J Ethnopharmacol*. 2004 Dec;95(2-3):287-296.

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	N/A
Activity:	agglutinates human ejaculated spermatozoa, but not human erythrocytes.
Inhibitory Carbohydrate:	N-acetyllactosamine (Gal beta 1,4GlcNAc), but is not significantly inhibited by monosaccharides or by lactose (Gal beta 1,4Glc), lacto-N-biose 1 (Gal beta 1,3GlcNAc), or chitobiose (GlcNAc beta 1,4GlcNAc).
Molecular Weight:	two different 12-kD lectin subunits for recombinant; 20kDa from affinity purified.

Ordering Information

Description	Cat. No.	Size
AMA affinity purified	L-8012-1	1 mg
AMA FITC	F-8012-1	1 mg
AMA TRITC	R-8012-1	1 mg
AMA Texas Red®	T-8012-1	1 mg
AMA Biotin	BA-8012-1	1 mg
AMA Horseradish Peroxidase	H-8012-1	1 mg
AMA Alkaline Phosphatase	LA-8012-1	1 mg
AMA Ferritin	I-8012-1	1 mg
AMA Colloidal Gold	GP-8012	1 ml
AMA Immobilized	A-8012-1	1 ml

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Dioclea grandiflora DGL (legume)

The lectin from the seeds of *Dioclea grandiflora* (DGL) is a Man/Glc-specific tetrameric protein with physical and saccharide-binding properties reported to be similar to that of the jack bean lectin concanavalin A (ConA). Unlike other plant lectins, both DGL and Con A bind with high affinity to the core trimannoside moiety, 3,6-di-O-(alpha-D-mannopyranosyl)-alpha-D-mannopyranoside, which is present in all asparagines-linked carbohydrates.¹ Unlike Con A, DGL does not bind to biantennary complex carbohydrates. This was confirmed by showing that biantennary complex glycopeptides do not bind to a DGL-Sepharose affinity column. Unlike ConA, DGL does not show enhanced affinity for a large N-linked oligomannose carbohydrate (Man9 glycopeptide) relative to the trimannoside. Thus, DGL and ConA share similar epitope recognition of the core trimannoside moiety. However they exhibit differences in their fine specificities for larger N-linked oligomannose and complex carbohydrates.¹ The overall structure of the DGL complex is similar to the structure of the ConA-trimannoside complex.² The location and conformation of the bound trimannoside as well as hydrogen-bonding interactions in both complexes are nearly identical. However, differences exist in the location of two loops outside of the respective binding sites containing residues 114-124 and 222-227. The latter residues affect the location of a network of hydrogen-bonded water molecules that interact with the trisaccharide. Differences in the arrangement of ordered water molecules in the binding site and/or protein conformational differences outside of the binding site may account for the differences in the thermodynamics of binding of the two lectins to deoxy analogs of the trimannoside. Molecular modeling studies suggest how DGL discriminates against binding the biantennary complex carbohydrate relative to ConA.³

C. bonariensis, *C. floribunda*, *D. rostrata*, and *D. violacea*, like *D. grandiflora*, show substantially reduced affinities for a biantennary complex carbohydrate with terminal GlcNAc residues, while *C. brasiliensis*, *D. guianensis*, and *D. virgata*, like ConA, exhibit affinities for the oligosaccharide comparable with that of the trimannoside. Thermodynamic data obtained by ITC indicate different energetic mechanisms of binding of the above two groups of lectins to the complex carbohydrate. The ability of the lectins to induce histamine release from rat peritoneal mast cells is shown to correlate with the relative affinities of the proteins for the biantennary carbohydrate.⁴

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2. Naismith, J.H., et al. (1996) *J. Biol. Chem.* **271**: 972-976.
3. Rozwarski, D.A., et al. (1998) *J. Biol. Chem.* **273**: 32818-32825
4. Dam, K., Cavada, B.S., et al. (1998) *J. Biol. Chem.* **273**: 12082-12088

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	Non-specific.
Activity:	Not tested.
Inhibitory Carbohydrate:	Mannose, Glucose.
Molecular Weight:	26-30 kDa

Ordering Information

Description	Cat. No.	Size
DGA affinity purified	L-9002-1	1 mg
DGA FITC	F-9002-1	1 mg
DGA TRITC	R-9002-1	1 mg
DGA Texas Red®	T-9002-1	1 mg
DGA Biotin	BA-9002-1	1 mg
DGA Horseradish Peroxidase	H-9002-1	1 mg
DGA Alkaline Phosphatase	LA-9002-1	1 mg
DGA Colloidal Gold	GP-9002	1 ml
DGA Immobilized	A-9002-1	1 ml

***Glechoma hederacea* GHA (ground ivy)**

A novel lectin has been isolated and cloned from the leaves of *Glechoma hederacea* (ground ivy), a typical representative of the plant family Lamiaceae. Biochemical analyses indicated that the *G. hederacea agglutinin* (Gleheda) is a tetrameric protein consisting of four subunits pairwise linked through an interchain disulphide bridge and exhibits a preferential specificity towards N-acetylgalactosamine. Cloning of the corresponding gene and molecular modeling of the deduced sequence demonstrated that Gleheda shares high sequence similarity with legume lectins and exhibits the same overall fold and three-dimensional structure as the classical legume lectins. The identification of a soluble and active legume lectin ortholog in *G. hederacea* not only indicates that the yet unclassified Lamiaceae lectins belong to the same lectin family as the legume lectins, but also sheds a new light on the specificity, physiological role and evolution of the classical legume lectins.¹

Screening of a population of wild plants revealed that Gleheda accounts for more than one-third of the total leaf protein in some clones, whereas it cannot be detected in other clones growing in the same environment. Gleheda is predominantly expressed in the leaves where it accumulates during early leaf maturation. The lectin is not uniformly distributed over the leaves but exhibits a unique localization pattern characterized by an almost exclusive confinement to a single layer of palisade parenchyma cells. Insect feeding trials demonstrated that Gleheda is a potent insecticidal protein for larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*). Because Gleheda is not cytotoxic, it is suggested that the insecticidal activity is linked to the carbohydrate-binding specificity of the lectin, which as could be demonstrated by agglutination assays with different types of polyagglutinable human erythrocytes is specifically directed against the Tn antigen structure (N-acetylgalactosamine O-linked to serine or threonine residues of proteins).²

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1. Wang, W., et al. (2002) *Plant Journal*. **33**: 293-304
2. Wang W, Hause B, et al. *Plant Physiol*. 2003 Jul;**132**(3):1322-34.

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	A > B, O.
Activity:	For trypsin treated human type A, B, and O, minimal concentration was 44, 176, and 708 ug/ml ⁻¹ respectively ¹ for hemagglutination.
Inhibitory Carbohydrate:	Gal, methyl- α -D-galactopyranoside and GalNAc.
Molecular Weight:	SDS-PAGE of unreduced Gleheda yielded a single polypeptide of approximately 60kDa whereas the reduced (with β -mercapto ethanol) protein yielded two polypeptide bands of 28 and 26kDa, which according to a densitometric analysis account for approximately 66 and 34% respectively of the total protein. ¹

Ordering Information

Description	Cat. No.	Size
GHA affinity purified	L-9003-1	1 mg
GHA FITC	F-9003-1	1 mg
GHA TRITC	R-9003-1	1 mg
GHA Texas Red®	T-9003-1	1 mg
GHA Biotin	BA-9003-1	1 mg
GHA Horseradish Peroxidase	H-9003-1	1 mg
GHA Alkaline Phosphatase	LA-9003-1	1 mg
GHA Ferritin	I-9003-1	1 mg
GHA Colloidal Gold	GP-9003	1 ml
GHA Immobilized	A-9003-1	1 ml

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***Hippeastrum hybrid* HHA (amaryllis)**

HHA reacts not only with terminal but also with internal alpha-D-mannosyl residues. Sugar hapten inhibition studies showed this lectin to possess the greatest specific activity for alpha-D-mannosyl units whereas D-Glc and D-GlcNAc did not inhibit the lectin precipitation system. Oligosaccharides containing either 1,3- or 1,6-linked mannosyl units were good inhibitors of the HHA-mannan precipitation system (6- to 20-fold more active than D-Man).(1)

Mannose-binding lectins, isolated from daffodil (NPA), amaryllis (HHA), and snowdrop (GNA) bulbs, are capable of precipitating with a linear mannopentaose (Man alpha 1-3Man alpha 1-3Man alpha 1-3Man alpha 1-2Man). NPA and HHA reacted strongly with the mannopentaose whereas GNA gave a precipitate only at concentrations greater than 500 microM. A phosphate group at C-6 of the nonreducing terminal mannosyl group prevented precipitation in all three cases. The reduced (NaBH₄) mannopentaose, Man₄Man-ol, did not precipitate with GNA or NPA, but was active with HHA. This activity was lost when Man₄Man-ol was converted (NaIO₄ then NaBH₄; mild acid hydrolysis of the reduced product) into trisaccharide derivatives.(2)

The plant lectins derived from *Galanthus nivalis* (Snowdrop) (GNA) and *Hippeastrum hybrid* (Amaryllis) (HHA) selectively inhibited a wide variety of human immunodeficiency virus type 1 (HIV-1) and HIV-2 strains and clinical (CXCR4- and CCR5-using) isolates in different cell types. They also efficiently inhibited infection of T lymphocytes by a variety of mutant virus strains. GNA and HHA markedly prevented syncytium formation between persistently infected HUT-78/HIV cells and uninfected T lymphocytes. The plant lectins did not measurably affect the antiviral activity of other clinically approved anti-HIV drugs used in the clinic when combined with these drugs. Short exposure of the lectins to cell-free virus particles or persistently HIV-infected HUT-78 cells markedly decreased HIV infectivity and increased the protective (microbicidal) activity of the plant lectins. Flow cytometric analysis and monoclonal antibody binding studies and a PCR-based assay revealed that GNA and HHA do not interfere with CD4, CXCR4, CCR5, and DC-SIGN and do not specifically bind with the membrane of uninfected cells. Instead, GNA and HHA likely interrupt the virus entry process by interfering with the virus envelope glycoprotein.

HHA and GNA are odorless, colorless, and tasteless, and they are not cytotoxic, antimetabolically active, or mitogenic to human primary T lymphocytes at concentrations that exceed their antivirally active concentrations by 2 to 3 orders of magnitude. GNA and HHA did not agglutinate human erythrocytes and were not toxic to mice when administered intravenously.(3)

REFERENCES

1. Kaku H, et al. Arch Biochem Biophys. 1990 Jun;279(2):298-304.
2. Kaku H, et al. Carbohydr Res. 1992 May 22;229(2):337-46.
3. Balzarini J, et al. Antimicrob Agents Chemother. 2004 Oct;48(10):3858-70.

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	N/A
Activity:	Does not agglutinate Human erythrocytes
Inhibitory Carbohydrate:	α ((1,3) or α ((1,6) linked mannosyl units
Molecular Weight:	A 50 kilodalton tetrameric lectin composed of four subunits of identical size. Also has an acidic isoelectric point.

Ordering Information

Description	Cat. No.	Size
HHA affinity purified	L-8008-1	1 mg
HHA FITC	F-8008-1	1 mg
HHA TRITC	R-8008-1	1 mg
HHA Texas Red®	T-8008-1	1 mg
HHA Biotin	BA-8008-1	1 mg
HHA Horseradish Peroxidase	H-8008-1	1 mg
HHA Alkaline Phosphatase	LA-8008-1	1 mg
HHA Ferritin	I-8008-1	1 mg
HHA Colloidal Gold	GP-8008	1 ml
HHA Immobilized	A-8008-1	1 ml

***Iris hybrid* IRA (Dutch Iris)**

A blood group type A disaccharide (GalNAc alpha 1-3Gal)-binding lectin has been purified from Dutch Iris (*Iris x hollandica*) bulbs. This lectin agglutinates both native and trypsin-treated rabbit erythrocytes, but not human erythrocytes, irrespective of blood group type. The lectin is a heterodimer consisting of two peptide chains (27 and 34 kDa) linked by disulfide bonds.

The carbohydrate binding specificity of the lectin was investigated by quantitative precipitation, hemagglutination inhibition, and precipitation inhibition assays. It is a Gal/GalNAc-specific lectin, with an extended carbohydrate combining site, which appears to be most complementary to GalNAc linked to the C-3 or C-6 hydroxyl group of galactose. As inhibitors, these disaccharides are approximately 30-60 times more potent than galactose and 4-8-fold more active than N-acetyl-D-galactosamine, whereas both the blood type A trisaccharide (GalNAc alpha 1-3[L-Fuc alpha 1-2]Gal) and the Forssman disaccharide (GalNAc alpha 1-3GalNAc) are noninhibitory, suggesting the importance of a free equatorial hydroxyl group at the C-2 position of the penultimate galactose for lectin binding; either an acetamido group or a fucosyl group at this position appears to cause steric hindrance, thus abolishing binding to the lectin.(1)

REFERENCES

1. Mo H, et al. J Biol Chem. 1994 Mar 11;269(10):7666-73.

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	N/A
Activity:	agglutinates native and trypsin-treated rabbit erythrocytes but not human erythrocytes.
Inhibitory Carbohydrate:	GalNAc alpha 1-3Gal beta-O
Molecular Weight:	Two peptide chains (27 and 34 kDa) linked by disulfide bonds.

Ordering Information

<u>Description</u>	<u>Cat. No.</u>	<u>Size</u>
IRA affinity purified	L-8010-1	1 mg
IRA FITC	F-8010-1	1 mg
IRA TRITC	R-8010-1	1 mg
IRA Texas Red®	T-8010-1	1 mg
IRA Biotin	BA-8010-1	1 mg
IRA Horseradish Peroxidase	H-8010-1	1 mg
IRA Alkaline Phosphatase	LA-8010-1	1 mg
IRA Ferritin	I-8010-1	1 mg
IRA Colloidal Gold	GP-8010	1 ml
IRA Immobilized	A-8010-1	1 ml

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Marasmius oreades agglutinin MOA (mushroom)

This mushroom lectin is highly specific for Gal α 1,3Gal and also recognizes the porcine xenotransplantation epitope (Gal α 1,3Gal β 1,4GlcNAc) and the blood group B determinant¹. This blood group B lectin, present in the mushroom *Marasmius oreades*, is available in its native and recombinant forms². It is an ideal reagent for the detection and identification of the Gal α 1,3Gal and Gal α 1,3Gal β 1,4GlcNAc/Glc epitopes present on chain ends of glycoproteins (e.g. porcine tissues and organs, porcine thyroglobulin, 3T3 cells, murine laminin, Ehrlich ascites tumor cells) and glycolipids³. It is greatly more specific for the above di- and tri-saccharides than the GS 1-B₄ isolectin which recognizes α -Gal end groups in any linkage. The lectin is available as a homogeneous, water-soluble protein, in its fluorescein- or biotinylated-forms or conjugated to agarose for use as an affinity matrix³.

In examining the binding of a series of analogues of the blood group B-trisaccharide, alphaGal(1-3)[alphaFuc(1-2)]betaGal-OR (1, R = (CH₂)₈COOMe), MOA was biotinylated and immobilized on a micro column (9.8 microL) for evaluation by Frontal Affinity Chromatography-Mass Spectrometry (FAC-MS) [2]. The trisaccharide 1 was found to be the epitope needed for maximum recognition (K_d = 3.6 microM). A series of synthetic deoxygenated and O-methylated analogues of the B-trisaccharide (R = OMe) were then screened against the lectin, and the key structural elements for binding were determined. OH-4 of the beta-Gal residue and OH-2 of the alpha-Gal residue were found to be critical for recognition.⁴

The binding properties of MOA and GS I-B(4) to the xenogenic disaccharide (Galalpha1-3Galbeta1) were comparable while the binding of MOA to the xenogenic pentasaccharide was much stronger than the binding of GS I-B(4) to the same epitope. Non-xenogenic disaccharide-coupled neoglycoproteins having galactose end groups linked alpha1-2 or alpha1-4 to Gal or linked alpha1-3 to GalNAc bound very weakly to MOA, whereas GS I-B(4) recognized all of these disaccharides with similarly high affinity. MOA also showed high affinity for laminin. The results indicate that the *Marasmius oreades* lectin has nearly the same affinities as does GS I-B(4) for the simple xenogenic carbohydrate antigens, but MOA has greater affinity for the pentasaccharide and is far more specific in its binding preferences than the Griffonia lectin.⁵

Investigation of the structure-function relationship of MOA defined the number and location of its carbohydrate-binding sites. Based on the sequence alignment of MOA to the ricin B-chain lactose-binding sites, we systematically constructed mutants by site-directed mutagenesis. Among amino acid residues at the putative carbohydrate-binding sites, Gln(46) in the alpha subdomain and Trp(138) in the gamma subdomain have been identified to be important amino acid residues directly or indirectly involved in carbohydrate recognition.

By surface plasmon resonance, Q46A and W138A were 2.4- and 4.3-fold less active than that of the wild-type MOA (K(a) = 2 x 10⁽⁷⁾), respectively. A double-site mutant (Q46A/W138A) had activity similar to W138A. The C-terminal deletion mutant MOADeltaC showed hemagglutination and precipitation activity, although its binding constant was 12.5-fold less active (K(a) = 1.6 x 10⁽⁶⁾) than that of the wild-type MOA. A C-terminal deletion mutant with mutations at both Gln(46) and Trp(138) (MOADeltaC-Q46A/W138A) was 12,500-fold less active (K(a) = 1.6 x 10⁽³⁾) than that of the wild-type MOA. Both alpha and gamma subdomains are most probably involved in carbohydrate binding, but the beta subdomain appears to be inactive.⁶

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1. Winter, H.C. et al. (2002). *J. Biochem.* **277**: 14996-15001.
2. Kruger, R.P., et al. (2002). *J. Biochem.* **277**: 15002-15005.
3. Teneberg, S., et al. (2003). *Glycobiology.* **13**: 479-486.
4. Rempel BP, Winter HC., et al. *Glycoconj J.* 2002 Mar;**19**(3):175-80.
5. Kirkeby S, et al, *Xenotransplantation.* 2004 May;**11**(3):254-61.
6. Tateno H, et al. *Arch Biochem Biophys.* 2004 Jul **427**(1):101-9.

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	B >> A
Activity:	Will agglutinate type B erythrocytes six times more avidly than type A.
Inhibitory Carbohydrate:	Gal α 1,3Gal and Gal α 1,3Gal β 1,4GlcNAc
Molecular Weight:	A heterodimeric protein of 50kDa, with subunits of 33 and 23 kDa.

Ordering Information

Description	Cat. No.	Size
MOA affinity purified	L-9001-1	1 mg
MOA FITC	F-9001-1	1 mg
MOA TRITC	R-9001-1	1 mg
MOA Texas Red®	T-9001-1	1 mg
MOA Biotin	BA-9001-1	1 mg
MOA Horseradish Peroxidase	H-9001-1	1 mg
MOA Alkaline Phosphatase	LA-9001-1	1 mg
MOA Colloidal Gold	GP-9001	1 ml
MOA Immobilized	A-9001-1	1 ml

Morniga M Morniga G MNA-G / MNA-M (black mulberry)

The bark of the black mulberry tree (*Morus nigra*) accumulates large quantities of a galactose-specific (Morniga G) and a mannose (Man)-specific (MornigaM) jacalin-related lectin. MornigaG resembles jacalin with respect to its molecular structure, specificity, and co- and posttranslational processing indicating that it follows the secretory pathway and eventually accumulates in the vacuolar compartment. In contrast, MornigaM represents a novel type of highly active Man-specific jacalin-related lectin that is synthesized without signal peptide or other vacuolar targeting sequences, and accordingly, accumulates in the cytoplasm. The isolation and cloning, and immunocytochemical localization of MornigaG and MornigaM not only demonstrates that jacalin-related lectins act as vegetative storage proteins in bark, but also allows a detailed comparison of a vacuolar galactose-specific and a cytoplasmic Man-specific jacalin-related lectin from a single species. Moreover, the identification of MornigaM provides the first evidence, to our knowledge, that bark cells accumulate large quantities of a cytoplasmic storage protein. In addition, due to its high activity, abundance, and ease of preparation, MornigaM is of great potential value for practical applications as a tool and bioactive protein in biological and biomedical research¹.

REFERENCES

1. Van Damme, Els, et al. (2002) *Plant Physiology*. **130** : 757-769

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	A.
Activity:	MNA-G has specific agglutination activity at 3.3 ng mL ⁻¹ . MNA-M has specific agglutination activity at 43 ng mL ⁻¹ .
Inhibitory Carbohydrate:	MNA-G is specific for Galactose. MNA-M is specific for Mannose.
Molecular Weight:	MNA-G has a MW=16,000 and MNA-M has a doublet of MW=15,000 and 16,000 when analyzed by SDS-PAGE. Higher molecular weight species (>5 million Da) are present in samples analyzed by gel filtration.

Ordering Information

Description	Cat. No.	Size
MNA-M affinity purified	L-9004-1	1 mg
MNA-M FITC	F-9004-1	1 mg
MNA-M TRITC	R-9004-1	1 mg
MNA-M Texas Red®	T-9004-1	1 mg
MNA-M Biotin	BA-9004-1	1 mg
MNA-M Horseradish Peroxidase	H-9004-1	1 mg
MNA-M Alkaline Phosphatase	LA-9004-1	1 mg
MNA-M Ferritin	I-9004-1	1 mg
MNA-M Colloidal Gold	GP-9004	1 ml
MNA-M Immobilized	A-9004-1	1 ml

Ordering Information

Description	Cat. No.	Size
MNA-G affinity purified	L-9005-1	1 mg
MNA-G FITC	F-9005-1	1 mg
MNA-G TRITC	R-9005-1	1 mg
MNA-G Texas Red®	T-9005-1	1 mg
MNA-G Biotin	BA-9005-1	1 mg
MNA-G Horseradish Peroxidase	H-9005-1	1 mg
MNA-G Alkaline Phosphatase	LA-9005-1	1 mg
MNA-G Ferritin	I-9005-1	1 mg
MNA-G Colloidal Gold	GP-9005	1 ml
MNA-G Immobilized	A-9005-1	1 ml

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***Polygonatum multiflorum* PMA (Solomon's seal)**

The most prominent protein of PMA is a mannose-binding lectin. It is a tetramer of four identical subunits of 14 kDa. Molecular modelling of the *Polygonatum* lectin and lectin-related protein indicated that the three-dimensional structure of both proteins strongly resembles that of the snowdrop lectin. (1)

Purified lectins agglutinated rabbit and rat erythrocytes best of all, they less agglutinated mice and horse erythrocytes, but did not agglutinate human, cow, sheep and frog erythrocytes at all. (2)

REFERENCES

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2. Antoniuk VO. *Ukr Biokhim Zh.* 1993 Jan-Feb;65(1):41-8.

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	N/A
Activity:	Agglutinates rabbit but not human erythrocytes.
Inhibitory Carbohydrate:	asialofetuin and asialomucin >> Native fetuin and mucin
Molecular Weight:	tetramer of four identical subunits of 14 kDa

Ordering Information

<u>Description</u>	<u>Cat. No.</u>	<u>Size</u>
PMA affinity purified	L-8009-1	1 mg
PMA FITC	F-8009-1	1 mg
PMA TRITC	R-8009-1	1 mg
PMA Texas Red®	T-8009-1	1 mg
PMA Biotin	BA-8009-1	1 mg
PMA Horseradish Peroxidase	H-8009-1	1 mg
PMA Alkaline Phosphatase	LA-8009-1	1 mg
PMA Ferritin	I-8009-1	1 mg
PMA Colloidal Gold	GP-8009	1 ml
PMA Immobilized	A-8009-1	1 ml

***Polyporus Squamosus* PSL (mushroom)**

A lectin from the fruiting body of the polypore mushroom *Polyporus squamosus* with an extended carbohydrate binding site that exhibits high specificity and affinity for non-reducing, terminal Neu5Ac α 2,6Gal β 1,4GlcNAc residues of N-linked glycans¹; it does not recognize α 2,3-linked sialic acid. Importantly, it does not recognize the Neu5Ac α 2,6GalNAc moiety present in ovine submaxillary mucin in contrast to the *S. nigra* agglutinin which requires only the disaccharide group². This indicates the necessity for three structural features for interaction with PSL. The lectin has been cloned and the recombinant lectin is available as a homogenous white powder. It is also available in its fluorescein- and biotinylated-forms as well as bound to agarose for use in affinity chromatography.

The native lectin, designated *P. squamosus* agglutinin, is composed of two identical 28kDa subunits associated by noncovalent bonds. *P. squamosus* agglutinin agglutinated human A, B, and O and rabbit red blood cells but precipitated only with human α_2 -macroglobulin, of many glycoproteins and polysaccharides tested.¹ Although the *P. squamosus* lectin binds β -D-galactosides, it has an extended carbohydrate-combining site that exhibits highest specificity and affinity toward non-reducing terminal Neu5Ac α 2,6Gal β 1,4Glc/GlcNAc(6'-sialylated type II chain) of N-glycans (2000-fold stronger than toward galactose). The strict specificity of the lectin for α 2,6-linked sialic acid renders this lectin a valuable tool for glycobiological studies in biomedical and cancer research.¹

cDNA cloning revealed that *P. squamosus* lectin contains a ricin B chain-like (QXW)₃ domain at its N-terminus that is composed of three homologous subdomains (α , β , and γ). A recombinant lectin was expressed in *Escherichia coli* as a fully active, soluble form. It agglutinated rabbit erythrocytes and showed greatest affinity for Neu5Ac α 2,6Gal β 1,4GlcNAc, but not the sialyl α 2,3-linked isomer. In investigations of the structure-function relationship of PSL, a monomeric C-terminal deletion mutant lacking 40% of the lectin's molecular weight retained sugar-binding activity, indicating that the carbohydrate-binding sites are situated in the N-terminal portion of the lectin, whereas the C-terminal portion possibly functions in oligomerization and structural stabilization. The EY Lectin is recombinantly-expressed and is a valuable reagent for the detection of Neu5Ac α 2,6Gal β 1,4GlcNAc sequence of asparagine-linked glycans.³

Product Characteristics

Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2-7.4.
Blood Group:	B > A,O.
Activity:	Not tested.
Inhibitory Carbohydrate:	Neu5Ac α 2,6Gal β 1,4GlcNAc.
Molecular Weight:	28kDa

Ordering Information

Description	Cat. No.	Size
PSL affinity purified	L-9006-1	1 mg
PSL FITC	F-9006-1	1 mg
PSL TRITC	R-9006-1	1 mg
PSL Texas Red®	T-9006-1	1 mg
PSL Biotin	BA-9006-1	1 mg
PSL Horseradish Peroxidase	H-9006-1	1 mg
PSL Alkaline Phosphatase	LA-9006-1	1 mg
PSL Ferritin	I-9006-1	1 mg
PSL Colloidal Gold	GP-9006	1 ml
PSL Immobilized	A-9006-1	1 ml

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1. Mo, H. et al. (2000) *J. Biol. Chem.* **275**:10623-10629.
2. Toma, V. et al. (2001) *Histochemistry* **116**: 183-193.
3. Tateno, H., Winter, H., et al. (2004) *Biochem J.* **382** (667-675)

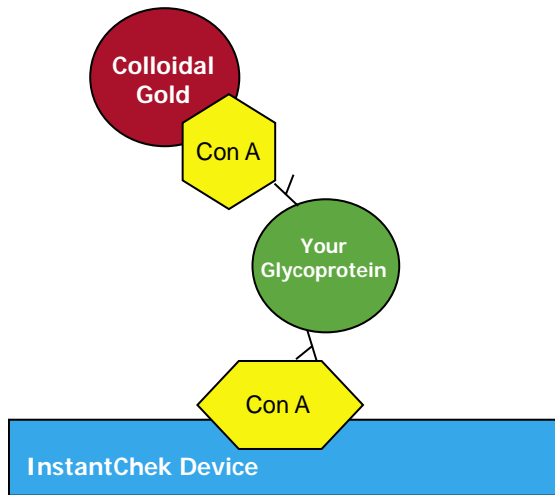
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InstantChek™ ONE-MINUTE KIT for Carbohydrate Identification

The InstantChek™ Lectin-Gold test kit is a simple, rapid sensitive specific test for rapid testing of carbohydrates that bind to lectins.

The lectin-gold conjugate is red in color and acts as a signal generator when bound to a specific carbohydrate of a glycoprotein or oligosaccharide.

Indirect Method



Indirect Method –

to detect a Carbohydrate of a Glycoprotein

A sandwich assay can be made of many different forms. For example, two of the same lectin can bind to a glycoprotein. Two different lectins may also bind to the same glycoprotein.

Preparation

To begin, select an InstantChek™ device pre-innoculated with lectin that is specific for the sugar you are detecting. For example, the Con A device is specific for Terminal Mannose or Branched Mannose.

Indirect Method Assay Protocol

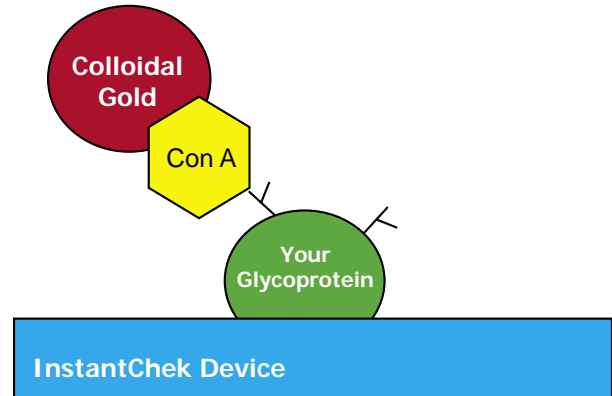
1. Add 10 µl Prewet solution. Let it absorb completely.
2. Add 5-10 µl Glycoprotein (1mg/ml). Let it absorb completely.
3. Add 50 µl Wash Buffer. Let it absorb completely.
4. Add 20 µl Reconstituted Lectin Gold that is specific for your carbohydrate. Let it absorb completely.
5. Wash by adding 50 µl Wash solution. Read Results.

Interpretation of Results

- Red to Pink dot indicates a Positive Result
- No dot indicates a Negative Result.

**CUSTOM KITS AVAILABLE
UPON REQUEST!**

Direct Method



Direct Method –

to detect a Carbohydrate of a Glycoprotein

Preparation

1. Select a blank device.
2. Inoculate 1-3 µg/µl of a glycoprotein at the center of the device membrane and let it air dry over one hour, depending on humidity conditions, or overnight. Once dry the device is now ready for the Assay Protocol.

Direct Method Assay Protocol

1. Add 10 µl Prewet solution to center of device. Let it absorb completely.
2. Add 20 µl of the reconstituted Lectin-Gold that is specific for your carbohydrate. Let it absorb completely.
3. Add 50 µl Wash Buffer. Read Results.

Kit Catalog No. IC-LG-001-5

Lectin-Gold	Carbohydrate Specificity
Con A	Terminal Mannose or Branched Mannose
WGA	GlcNAc or Sialic Acid
SBA	Gal A or B – linked
UEA-I	L-Fucose alpha linked
DBA	GalNAc alpha-linked

Kit Contents

10 Units (2x5 strips) Pre-Innoculated Con A InstantChek Device
 10 Units (2x5 strips) Pre-Innoculated WGA InstantChek Device
 10 Units (2x5 strips) Pre-Innoculated SBA InstantChek Device
 10 Units (2x5 strips) Pre-Innoculated UEA-I InstantChek Device
 10 Units (2x5 strips) Pre-Innoculated DBA InstantChek Device

10 Units (2x5 strips) Blank InstantChek Device

Reagents:

3 x 5 Different lectin-gold conjugates in lyophilized form.
 (Con A, WGA, UEA-I, SBA, DBA)
 1x 5ml Prewet Solution
 1x 10ml Reconstitution Buffer
 2x 10ml Wash Buffer
 1x 1 InstantChek™ Lectin-Gold test kit insert

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