A Comprehensive Toolkit of Plant Cell Wall Glycan-Directed Monoclonal Antibodies

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SUPPLEMENTAL MATERIALS

Supplemental Methods:

Hierarchical clustering of mAbs. This clustering analysis was tailored for comparison of the ELISA responses among the mAbs when tested against the panel of polysaccharides. A matrix of ELISA data was generated, with each row vector of this matrix consisting of the raw ELISA response vector for a particular mAb assayed against the set of polysaccharides. Each row vector was normalized to unit length and then a correlation matrix was generated by calculating the dot product of each pair of rows in the normalized data matrix. Thus, each element of the correlation matrix corresponds to the cosine of the arc (on the surface of a unit hypersphere) connecting two of the normalized row vectors in the data matrix. A distance matrix was generated based on the arccosines of the elements of the correlation matrix. Hierarchical clustering was performed by calling the R function hclust using this distance matrix as its argument to generate a cluster matrix, which was used to generate a mAb dendrogram. A similar process was used to generate a polysaccharide dendrogram, using the columns of the normalized data matrix as the initial input. The R heatmap function was modified and used to create a heat map, using the two dendrograms and the initial data matrix as inputs (Figure 2).

To compare experimental replicates of the ELISA data sets, "data correlation heat maps" were generated. Two raw data matrices (one for the reference data set and one for the test data set) were generated as described above. The rows of the reference matrix were clustered and a dendrogram of mAb ELISA response patterns was generated as described above. Then, a different kind of correlation matrix, in which each element consists of the dot product of a row vector in the test matrix with a row vector in the reference matrix, was generated (Figure 1). This correlation matrix was plotted using a modification of the R heatmap function with the mAb dendrogram for the reference data set and the correlation matrix as input arguments. In this heat map, the order of rows (mAbs in the reference matrix) and columns (mAbs in the test matrix) is the same, both being based on the dendrogram generated by clustering rows of ELISA data in the reference matrix. Each cell on the diagonal of this heat map (*i.e.*, each element on the diagonal of the correlation matrix) thus corresponds to the correlation of two independent experimental measurements of the ELISA response pattern for a particular mAb. Each offdiagonal element in the correlation matrix corresponds to the correlation of the ELISA response pattern for a mAb (*i.e.*, row vector *i* from one experiment) with the ELISA response pattern for another mAb (row vector *j* from another experiment). Perfectly reproducible data corresponds to

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the situation where response vectors i and j in the reference matrix are equal to the corresponding response vectors in the test matrix. This would result in a correlation matrix (heat map) in which all diagonal elements have a value of one. This matrix would also be symmetrical about the diagonal, as the correlation of row vector i in the reference matrix with row vector j in the test matrix would be equal to the correlation of row vector j in the reference matrix with row vector i in the test matrix.



Figure S1. Suitability of 96-well plates for polysaccharide ELISAs.

Twelve mAb/polysaccharide pairs (see Materials and Methods) were tested on eight commercially available 96-well plates to determine which plates are best suited for immobilization of wall polysaccharides and subsequent ELISAs. Bars indicate the OD value given by each mAb when tested in the ELISA against a polysaccharide that mAb is known to recognize.

Table S1: Plant polysaccharide preparations used in this study.

Polysaccharide preparations are grouped with respect to the predominant glycan component. Information is provided as to the plant origin of the preparation, abbreviation used in the paper, source and sugar composition.

Polysaccharide class	Preparation (Abbreviation)	Source and reference(s)/website for structural studies	Sugar composition (Mol %)
	Tamarind Xyloglucan (Tam XG)	CCRC, UGA; (York et al., 1993), Hahn lab data (unpublished)	Glc (43), Xyl (37), Gal (17) and Ara (3)
XGs	Tomato Xyloglucan (Tom XG)	CCRC, UGA; (Jia et al., 2003), Hahn lab data (unpublished)	Glc (44), Xyl (32), Gal (14) and Ara (10)
	Sycamore Maple Xyloglucan (Syc XG)	CCRC, UGA; (Stevenson et al., 1986), Hahn lab data (unpublished)	Glc (42), Xyl (31), Gal (10), Man (9), Fuc (4) and Ara (4)
	Wheat Arabinoxylan (Wh Ara Xyl)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-WAXYL.pdf	Ara (37), Xyl (61) and traces of other sugars
	4-O-Methylglucurono- xylan (MeGLA Xyl)	Sigma-Aldrich, St. Louis, MO; Hahn lab data (unpublished)	Xyl (82.8) and Methyl GIcA (17.2)
	Birch Wood Xylan (BW Xyl)	Sigma-Aldrich, St. Louis, MO; Hahn lab data (unpublished)	Xyl (100)
	Phormium tenax Partially Hydrolysed Xylan (PT Xyl PH)	IRL, New Zealand; Hahn lab data (unpublished)	Xyl (94) and Gluc (6)
Xylans	Phormium tenax Low Arabinose Xylan (PT Xyl LA)	IRL, New Zealand; Hahn lab data (unpublished)	Xyl (87), Ara (8) and Glc (5)
	Phormium cookianum High Arabinose Xylan (PC Xyl)	IRL, New Zealand; Hahn lab data (unpublished)	Xyl (70), Ara (27) and Glc (3)
	Corn Xylan (Corn Xyl)	National Renewable Energy Lab, Golden, CO; Hahn lab data (unpublished)	ХуІ (100)
	Poplar Xylan (Pop Xyl)	National Renewable Energy Lab, Golden, CO; Hahn lab data (unpublished)	Xyl (94), GalA (4) and Rha (2)
	Eucalyptus KOHss (Eucal KOH)	Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen, The Netherlands; personal communication	Xyl (81), UA (13), Gal (3), Rha (2), Ara (1), Glc (1) and Man (1)
	Sorghum BE1 (Sorg BE1)	Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen, The Netherlands; personal communication	Ara (45.8), Xyl (40.9), UA (9.8), Gal (1.8), Gic (1.7) and Man (0.2)
	Corn BE1 (Corn BE1)	Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen, The Netherlands; personal communication	Ara (38.4), Xyl (48.3), UA (8.3), Gal (4.3), Glc (0.7) and Man (0.1)
	Tomato Glucomannan (Tom Gluc Man)	CCRC, UGA; Hahn lab data (unpublished)	Glc (61) and Man (39)
Mannans	Guar Galactomannan (Guar Gal Man)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-GGMMV.pdf	Man (62) and Gal (38)
	Gum Guar (Gum Guar)	Sigma-Aldrich, St. Louis, MO; Hahn lab data (unpublished)	Man (63), Gal (36) and Ara (1)
	Locust Bean Gum (Loc Bean Gum)	Sigma-Aldrich, St. Louis, MO; Hahn lab data (unpublished)	Man (81) and Gal (19)

	(1→3)(1→4)-β-Glucan (1314 Gluc)	Megazyme, Bray, Ireland; Hahn lab data (unpublished); Hahn lab data (unpublished)	Glc (97), Man (2) and Ara (1)
R Chucopa	(1→3)(1→6)-β-Glucan (1316 Gluc)	CCRC, UGA; (Hahn et al., 1992), Hahn lab data (unpublished)	Glc (91), Man (7) and Ara (2)
p-Glucans	Pachyman (Pachyman)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-PACHY.pdf	D-glucose essentially all of which is 1,3-ß-linked (>98)
	Lichenan (Lichenan)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-LICHN.pdf	Glc (98) and Ara (2)
	Lupin Galactan (Lup Gal)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-GALLU.pdf	Gal (83), GalA (5), Rha (5), Ara (3), Xyl (2) and traces of Glc
Galactans	Potato Galactan (Pot Gal)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P- GALPOT.pdf	Gal (88), GalA (6), Ara (3) and Rha (3)
	Larch Arabinogalactan (Lar Ara Gal)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-ARGAL.pdf	Gal (81), Ara (14) and traces of other sugars
	Gum Arabic (Gum Arabic)	Sigma-Aldrich, St. Louis, MO; (Stephen, Phillips, and Williams, 2006); Hahn lab data (unpublished)	Ara (37), Gal (40), Rha (20) and traces of Glc
	Gum Ghatti (Gum Ghatti)	Sigma-Aldrich, St. Louis, MO; (Stephen, Phillips, and Williams, 2006); Hahn lab data (unpublished)	Ara (49), Gal (31), Man (10), GlcA (8) and Xyl (2)
	Gum Tragacanth (Gum Trag)	Sigma-Aldrich, St. Louis, MO; (Stephen, Phillips, and Williams, 2006); Hahn lab data (unpublished)	Gal (32.3), Ara (31.7), Xyl (12), Glc (12.3), Fuc (8.4) and Rha (3.3)
	Arabinan (Sug Beet MGZ Branch Arab)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-ARAB.pdf	Ara (88), Gal (3), Rha (2) and GalA (7)
AGs	Linear Arabinan (Sug Beet MGZ Lin Arab)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-LARB.pdf	Ara (97.5), GalA (2), Gal (0.4) and Rha (0.1)
	Sugar Beet Linear Arabinan (Sug Beet Lin Arab)	Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen, The Netherlands; personal communication	Ara (74), UA (14) and Gal (12)
	Sugar Beet Branched Arabinan (Sug Beet Branch Arab)	Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen, The Netherlands; personal communication	Ara (>80)
	Potato Arabinogalactan (Pot Ara Gal)	Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen, The Netherlands; personal communication	Gal (63), Ara (20) and UA (17)
	Arabinogalactan II (Ara Gal II)	Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen, The Netherlands; personal communication	Gal (84) and Gal (16)
	Sycamore Maple Pectic Polysaccharides (Syc PecP)	CCRC, UGA; (Stevenson et al., 1986); Hahn lab data (unpublished)	Gal (52.4), GlcA (15), GalA (11), Xyl (7.9), Ara (7.5), Glc (2.6), Rha (2), Man (1.3) and traces of Fuc
	Tomato Pectic Polysac- charides (Tom PecP)	CCRC, UGA; (Jia et al., 2003); Hahn lab data (unpublished)	Gal (57.4), Ara (25.2), GalA (6.5), Rha (4.4), Xyl (4.3) and Fuc (2.2)
RG-I	Physcomitrella patens Pectic Polysaccharides (PhyscPecP)	CCRC, UGA; (Peña et al., 2008)	GalA (46.7), Rha (24), Gal(17.4), Ara (8.1), Glc (2.3) and 1.5 (Xyl)
	Gum Karaya (Gum Karaya)	Sigma-Aldrich, St. Louis, MO; Hahn lab data (unpublished)	GalA (57), GlcA (8), Gal (17), Rha (16) and traces of Glc
	Potato RG-I (Pot RG-I)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-RHAM1.pdf	GalA (51), Rha (28.6), Gal (14), Ara (5.4) and Xyl (1)

	Soybean RG-I (Soy RG-I)	Megazyme, Bray, Ireland; Hahn lab data (unpublished), http://secure.megazyme.com/downloads/en/data/P-RHAGN.pdf	GalA (51), Xyl (13), Gal (11), Rha (10), Fuc (9) and Ara (6)
	Arabidopsis thaliana RG-I (At RG-I)	CCRC, UGA; (Zablackis et al., 1995)	GalA (34), Rha (27), Gal (17), Ara (14), Xyl (5), Fuc (1), GlcA (1) and GalA (1)
	Green Tomato Fruit RG-I (GrTomFrRG-I)	CCRC, UGA; Hahn lab data (unpublished)	GalA (49.5), Gal (43.8), Rha (4.7), GlcA (1), Ara (0.5) and traces of Xyl and Glc
	Lettuce RGI (Let RG-I)	CCRC, UGA; Hahn lab data (unpublished)	Gal (54.9), GalA (23.4), Rha (6.9), GlcA (5.7), Ara (4.9) and Xyl (4.2)
	RG-I from Okra (Okra RG-I)	Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen, The Netherlands; personal communication	Gal (53), GalA (26), Rha (14), GlcA (4), Glc (2.5) Ara (1) and Man (1)
	Arabidopsis thaliana Seed Mucilage (At Seed Muc)	CCRC, UGA; Hahn lab data (unpublished)	Rha (69), GalA (27.7), Gal (1.8) and Xyl (1.5)
Mucilages	Sinapus Seed Mucilage (Sin Seed Muc)	CCRC, UGA; Hahn lab data (unpublished)	GalA (57), Gal (19), Rha (12), GlcA (10) and traces of Man and Glc
	Peppergrass Seed Mucilage (Pep Gr S Muc)	CCRC, UGA; Hahn lab data (unpublished), (Deng et al., 2009)	GlcA (22.2), GalA (22.1), Gal (18.9), Xyl (9.8), Rha (8), Glc (7), Fuc (5.2), Ara (5) and Man (1.8)
	Camelina Seed Mucilage (Cam Seed Muc)	CCRC, UGA; Hahn lab data (unpublished)	Rha (57.1), GalA (24.4), Gal (13.3), Xyl (2.2), Ara (2.3) and Man (0.8)
	Linseed Mucilage (Lin Seed Muc)	CCRC, UGA; Hahn lab data (unpublished)	Rha (44.2), GalA (18.5), Xyl (15.8), Gal (11.1), Fuc (3), Ara (4.6) and Glc (2.9)
	Citrus Pectin (Citrus Pect)	Hercules, Wilmington, DE; Hahn lab data (unpublished)	GalA (85), Rha (13) and Gal (2)
	73% Me Pectin (73%MePect))	Hercules, Wilmington, DE; Hahn lab data (unpublished)	GalA (81), Gal (7), Rha (6.5), Ara (4.6) and Glc (0.9)
HGs	55% Me Pectin (55%MePect)	Hercules, Wilmington, DE; Hahn lab data (unpublished)	GalA (81), Gal (10), Rha (9)
	33% Me Pectin (33%MePect)	Hercules, Wilmington, DE; Hahn lab data (unpublished)	GalA (89), Gal (7), Rha (4)
	High-acetyl Sugar Beet β-Pectin (HAc Sug Beet Pect)	Hercules, Wilmington, DE; Hahn lab data (unpublished)	GalA (56.2), Rha (21.3), Gal (15) and Ara (7.5)

Table S2: Monoclonal antibodies included in this study.

McAbs are grouped according to the major glycan recognized. The order of the antibodies within each group arises out of the hierarchical clustering analysis of the polysaccharide screening ELISA data (Figure 2). Information is provided for each McAb as to the immunogen used to generate the antibody, animal source, isotype (including light chain specifications) and references.

Glycans	Antibody	Immunogen	Animal	Isotype	References
	CCRC-M54	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M48	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M49	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M96	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG3(λ)	
	CCRC-M50	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M51	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M53	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	LM15	XXXG-BSA (covalent)	Rat	lgG2c(κ)	(Marcus et al., 2008)
	CCRC-M100	Sycamore Xyloglucan-BSA (covalent)	Mouse	lgM(κ)	
U U	CCRC-M103	Sycamore Xyloglucan-BSA (covalent)	Mouse	lgM(κ)	
(pé	CCRC-M58	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(κ)	
late	CCRC-M86	Tomato Xyloglucan-BSA (covalent)	Mouse	lgM(κ)	
Non-fucosyl	CCRC-M55	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M52	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M99	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M95	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M101	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M104	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M89	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M93	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M87	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M88	Tomato Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M57	Tamarind Xyloglucan-BSA (covalent)	Mouse	lgG1(λ)	
	CCRC-M90	Tomato Xyloglucan-BSA (covalent)	Mouse	lgM(λ)	
	CCRC-M47	Arabidopsis thaliana Cell Wall	Mouse	lgM(λ)	

	CCRC-M111	Phormium tenax xylan (low arabinose)/MeBSA	Mouse	lgG1(λ)	
Xylan-1	CCRC-M108	Phormium tenax xylan (low arabinose)/MeBSA	Mouse	lgG1(λ)	
	CCRC-M109	Phormium tenax xylan(low arabinose)/MeBSA	Mouse	lgG1(λ)	
	CCRC-M102	Sycamore Xyloglucan-BSA (covalent)	Mouse	lgM(κ)	
Evenevilated	CCRC-M39	4-O-methylglucuronoxylan (Sigma)/MeBSA	Mouse	lgA(κ)	
Fycosylated	CCRC-M106	Sycamore Xyloglucan-BSA (covalent)	Mouse	lgG1(κ)	
ΛG	CCRC-M84	Sycamore Pectic Polysaccharides/MeBSA	Mouse	lgM(κ)	
	CCRC-M1	Sycamore RG-I/MeBSA	Mouse	lgG1(κ)	(Puhlmann et al., 1994)
Undefined	CCRC-M76	Sycamore Pectic Polysaccharides/MeBSA	Mouse	lgG1(κ)	
	JIM3	Carrot Protoplasts	Rat	lgM(κ)	(K. Roberts, pers. comm.)
ge	CCRC-M40	Arabidopsis thaliana Cell Wall	Mouse	IgM(κ)	
cila	CCRC-M83	Arabidopsis thaliana RG-II/MeBSA	Mouse	lgM(κ)	
ли Чи	CCRC-M82	Arabidopsis thaliana RG-II/MeBSA	Mouse	lgM(κ)	
L DR	MH4.4E4	Tobacco (Nicotiana tabacum) Leaf Protoplasts	Mouse	lgM(κ)	(Hahn et al., 1987)
see	MH4.2A4	Tobacco (Nicotiana tabacum) Leaf Protoplasts	Mouse	lgM(κ)	(Hahn et al., 1987)
.E CCRC-M14		Linseed Mucilage RGI/MeBSA	Mouse	lgM(κ)	
	CCRC-M141	Linseed Mucilage RGI/MeBSA	Mouse	lgG2b(κ)	
	CCRC-M119	Phormium cookianum xylan (high arabinose)/MeBSA	Mouse	lgM(κ)	
	CCRC-M115	Phormium cookianum xylan (high arabinose)/MeBSA	Mouse	lgG1(κ)	
	CCRC-M110	Phormium tenax xylan (low arabinose)/MeBSA	Mouse	lgG1(λ)	
	CCRC-M105	Phormium tenax xylan (low arabinose)/MeBSA	Mouse	lgA(κ)	
	CCRC-M117	Phormium cookianum xylan (high arabinose)/MeBSA	Mouse	lgG1(κ)	
Vulan 2	CCRC-M113	Phormium cookianum xylan (high arabinose)/MeBSA	Mouse	lgG1(κ)	
	CCRC-M120	Phormium cookianum xylan (high arabinose)/MeBSA	Mouse	lgM(κ)	
	CCRC-M118	Phormium cookianum xylan (high arabinose)/MeBSA	Mouse	lgM(κ)	
	CCRC-M116	Phormium tenax xylan (low arabinose)/MeBSA	Mouse	lgM(κ)	
	CCRC-M114	Phormium cookianum xylan (high arabinose)/MeBSA	Mouse	lgM(κ)	
	CCRC-M154	Corn Stover Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M150	Corn Stover Xylan-BSA (covalent)	Mouse	lgG3(κ)	
Undefined	MAC265	Infection Thread Matrix Glycoproteins	Rat	lgG2a(κ)	(VandenBosch et al., 1989)
Undenned	CCRC-M97	Physcomitrella patens Pectic Polysaccharides/MeBSA	Mouse	lgA(κ)	

Physcomitrella	CCRC-M98	Physcomitrella patens Pectic Polysaccharides/MeBSA	Mouse	lgG1(κ)	
Pectin	CCRC-M94	Physcomitrella patens Pectic Polysaccharides/MeBSA	Mouse	lgM(κ)	
	CCRC-M5	Sycamore RG-I/MeBSA	Mouse	lgG1(κ)	(Puhlmann et al., 1994)
RG-la	CCRC-M2	Sycamore RG-I/MeBSA	Mouse	lgM(κ)	(Puhlmann et al., 1994)
	CCRC-M23	Wine RG-II-cBSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M17	Sycamore RG-II-cBSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M19	Sycamore RG-II-cBSA (covalent)	Mouse	lgM(κ)	
KG-ID	CCRC-M18	Sycamore RG-II-cBSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M56	Soybean RG-I (Megazyme)/MeBSA	Mouse	lgG1(κ)	
	CCRC-M16	Sycamore RG-II-cBSA (covalent)	Mouse	lgG1(κ)	
	JIM137	Feruloylated Sugarbeet Cell Wall Pectins	Rat	lgM(κ)	(K. Roberts, pers. comm.)
	JIM101	Liverwort (Gymnocolea inflata) AGP	Rat	lgM(κ)	(K. Roberts, pers. comm.)
RG-Ic	CCRC-M135	Camelina sativa Seed Mucilage/MeBSA	Mouse	lgA(κ)	
	CCRC-M61	Sinapus Seed Mucilage/MeBSA	Mouse	lgM(κ)	
	CCRC-M30	Arabidopsis thaliana Seed Mucilage/MeBSA	Mouse	lgM(κ)	
Colorto	CCRC-M75	Guar Galactomannan (Megazyme)-BSA (covalent)	Mouse	lgM(κ)	
Galacto-	CCRC-M70	Guar Galactomannan (Megazyme)-BSA (covalent)	Mouse	lgM(κ)	
mannan	CCRC-M74	Guar Galactomannan (Megazyme)-BSA (covalent)	Mouse	lgM(κ)	
β-1,3 Glucan	β-1,3 Glucan LAMP Laminarin-Haemocyanin (covalent)		Mouse	lgG1(κ)	(Meikle et al., 1991)
	JIM93	Pea Protoplast Membrane	Rat	lgG2c(κ)	(K. Roberts, pers. comm.)
	JIM94	Pea Protoplast Membrane	Rat	lgG2c(κ)	(K. Roberts, pers. comm.)
AG-1	JIM11	Carrot Cell Nuclear Matrix Protein Extract	Rat	lgG2c(κ)	(Smallwood et al., 1994)
	MAC204	Pea Peribacteroid Membrane	Mouse	lgM(κ)	(Bradley et al., 1988)
	JIM20	Pea Guard Cell Protoplasts	Rat	lgM(κ)	(Smallwood et al., 1994)
	LM6	Arabinoheptaose-BSA (covalent)	Rat	lgG2c(κ)	(Willats et al., 1998)
	JIM14	Carrot AGP2	Rat	lgM(κ)	(Knox et al., 1991)
	MAC207	Pea Peribacteroid Membrane	Mouse	lgM(κ)	(Bradley et al., 1988)
AC 2	JIM19	Pea Guard Cell Protoplasts	Rat	lgM(κ)	(Wang et al., 1995)
AG-2	JIM12	Carrot Cell Nuclear Matrix Protein Extract	Rat	lgM(κ)	(Smallwood et al., 1994)
	CCRC-M133	Citrus Pectin/MeBSA	Mouse	lgM(κ)	
	CCRC-M107	Arabidopsis thaliana RG-II/MeBSA	Mouse	lgM(κ)	
	LM5	Galactotetraose-BSA (covalent)	Rat	lgG2c(κ)	(Jones et al., 1997)

Lindofined	CCRC-M136	Peppergrass Seed Mucilage/MeBSA	Mouse	lgG1(κ)	
Undenned	LM8	Xylogalacturonan/MeBSA	Rat	lgM(κ)	(Willats et al., 2004)
	JIM4	Carrot Protoplasts	Rat	lgM(κ)	(Knox and Roberts, 1989)
	CCRC-M31	Arabidopsis thaliana Seed Mucilage/MeBSA	Mouse	lgM(κ)	
	JIM17	Carrot Extracellular Glycoproteins	Rat	lgM(κ)	(K. Roberts, pers. comm.)
	LM1	Rice Hydroxyproline-Rich Glycoproteins (Extensin)	Rat	lgM(κ)	(Smallwood et al., 1995)
	CCRC-M26	Phytophthora Void Glucan-BSA (covalent)	Mouse	lgA(κ)	
AG-3	JIM15	Carrot AGP1	Rat	lgM(κ)	(Knox et al., 1991)
	JIM8	Sugarbeet Suspension-Cultured Protoplasts	Rat	lgG2c(κ)	(Pennell et al., 1991)
	CCRC-M85	Sycamore Pectic Polysaccharides/MeBSA	Mouse	lgM(κ)	
	CCRC-M81	Sycamore Pectic Polysaccharides/MeBSA	Mouse	lgM(κ)	
	MAC266	Golgi Glycoproteins	Rat	lgG2a(κ)	(Perotto et al., 1991)
	PN16.4B4	Tobacco (Nicotiana glutinosa) Cell Membranes	Mouse	lgM(κ)	(Norman et al., 1986)
	CCRC-M132	Peppergrass Seed Mucilage/MeBSA	Mouse	lgG1(κ)	
	CCRC-M131	Peppergrass Seed Mucilage/MeBSA	Mouse	lgG1(κ)	
	CCRC-M38	Arabidopsis thaliana Seed Mucilage/MeBSA	Mouse	lgG1(κ)	
	JIM5	Carrot Protoplasts	Rat	lgG2a(κ)	(Knox et al., 1990; VandenBosch et al., 1989)
Ð	PAM1	Not applicable	Human	not applicable	(Willats et al., 1999)
Lo	CCRC-M69	Sinapus Seed Mucilage/MeBSA	Mouse	lgM(κ)	
R K C	CCRC-M35	Arabidopsis thaliana Seed Mucilage/MeBSA	Mouse	lgM(κ)	
Ba	CCRC-M36	Arabidopsis thaliana Seed Mucilage/MeBSA	Mouse	lgM(κ)	
ţi	CCRC-M14	Sycamore RG-I/MeBSA	Mouse	lgM(κ)	
ec	CCRC-M129	Citrus Pectin /MeBSA	Mouse	lgG1(κ)	
L.	CCRC-M72	Sinapus Seed Mucilage/MeBSA	Mouse	lgA(κ)	
	LM7	Lime pectin	Rat	lgM(κ)	(Willats et al., 2001)
	CCRC-M34	Arabidopsis thaliana Seed Mucilage/MeBSA	Mouse	lgM(λ)	
	CCRC-M130	Heat-treated Citrus Pectin	Mouse	lgG1(κ)	
	JIM136	Feruloylated Sugarbeet Cell Wall Pectins	Rat	lgM(κ)	(K. Roberts, pers. comm.)
	JIM7	Carrot Protoplasts	Rat	lgA(κ)	(Knox et al., 1990)

	PN16.1B3	Tobacco (Nicotiana glutinosa) Cell Membranes	Mouse	lgM(κ)	(Norman et al., 1986)
	CCRC-M124	RG-I pentamer-KLH (covalent)	Mouse	lgG1(κ)	
	JIM133	Zinnea Tracheary Element Cell Walls	Rat	lgM(κ)	(K. Roberts, pers. comm.)
AG-4	JIM13	Carrot AGP2	Rat	lgM(κ)	(Knox et al., 1991)
	LM2	Rice Arabinogalactan Proteins	Rat	lgM(κ)	(Smallwood et al., 1996)
	CCRC-M92	Sycamore Pectic Polysaccharides/MeBSA	Mouse	lgM(κ)	
	CCRC-M91	Sycamore Pectic Polysaccharides/MeBSA	Mouse	lgM(κ)	
	CCRC-M78	Arabidopsis thaliana EPG RG-I/MeBSA	Mouse	lgM(κ)	
	CCRC-M60	Sinapus Seed Mucilage/MeBSA	Mouse	IgM(κ)	
	CCRC-M41	Arabidopsis thaliana Cell Wall	Mouse	lgM(κ)	
	CCRC-M80	Arabidopsis thaliana RG-II/MeBSA	Mouse	lgG1(κ)	
	CCRC-M79	Arabidopsis thaliana EPG RG-I/MeBSA	Mouse	lgG1(κ)	
	CCRC-M44	Arabidopsis thaliana Cell Wall	Mouse	lgM(κ)	
	CCRC-M33	Arabidopsis thaliana Seed Mucilage/MeBSA	Mouse	lgM(κ)	
	CCRC-M32	Arabidopsis thaliana Seed Mucilage/MeBSA	Mouse	lgM(κ)	
	CCRC-M13	Sycamore RG-I/MeBSA	Mouse	lgG1(κ)	
	CCRC-M42	Arabidopsis thaliana Cell Wall	Mouse	lgM(κ)	
	CCRC-M24	Wine RG-II-cBSA (covalent)	Mouse	lgM(κ)	
	CCRC-M12	Sycamore RG-I/MeBSA	Mouse	lgG1(κ)	(Puhlmann et al., 1994)
PG I/AG	CCRC-M7	Sycamore RG-I/MeBSA	Mouse	lgG1(κ)	(Puhlmann et al., 1994)
NG-I/AG	CCRC-M77	Arabidopsis thaliana EPG RG-I/MeBSA	Mouse	lgG1(κ)	
	CCRC-M25	Wine RG-II-cBSA (covalent)	Mouse	lgM(κ)	
	CCRC-M9	Sycamore RG-I/MeBSA	Mouse	lgM(κ)	
	CCRC-M128	Citrus Pectin /MeBSA	Mouse	lgM(κ)	
	CCRC-M126	RG-I pentamer-KLH (covalent)	Mouse	lgG3(κ)	
	CCRC-M134	Citrus Pectin/MeBSA	Mouse	lgG3(κ)	
	CCRC-M125	RG-I pentamer-KLH (covalent)	Mouse	lgM(κ)	
	CCRC-M123	RG-I pentamer-KLH (covalent)	Mouse	lgG1(κ)	
	CCRC-M122	RG-I pentamer-KLH (covalent)	Mouse	lgG1(κ)	
	CCRC-M121	RG-I pentamer-KLH (covalent)	Mouse	lgG1(κ)	
	CCRC-M112	Arabidopsis thaliana RG-II/MeBSA	Mouse	lgG3(κ)	
	CCRC-M21	Sycamore RG-I (0.4% Ara)/MeBSA	Mouse	lgG3(κ)	

	JIM131Zinnea Tracheary Element Cell WallsCCRC-M22Sycamore RG-I (0.4% Ara)/MeBSA		Rat	lgG1(κ)	(K. Roberts, pers. comm.)
			Mouse	lgG1(κ)	
	JIM132	Zinnea Tracheary Element Cell Walls	Rat	lgM(κ)	(K. Roberts, pers. comm.)
	JIM1	Carrot Protoplasts	Rat	lgG2c(κ)	(K. Roberts, pers. comm.)
	CCRC-M15	Sycamore RG-I/MeBSA	Mouse	lgM(κ)	
	CCRC-M8	Sycamore RG-I/MeBSA	Mouse	lgM(κ)	
	MH4.3E5	Tobacco (Nicotiana tabacum) Leaf Protoplasts	Mouse	lgM(κ)	(Hahn et al., 1987)
	JIM16	Carrot AGP1	Rat	lgM(κ)	(Knox et al., 1991)
	CCRC-M160	Corn Cob Red-BSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M137	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M152	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M149	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
Vulan 2	AX1	Oligo-arabinoxylan-BSA (covalent)	Mouse	lgG1(λ)	(Guillon et al., 2004)
Aylan-3	CCRC-M144	Corn Stover Xylan-BSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M143	Corn Stover Xylan-BSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M146	Corn Stover Xylan-BSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M145	Corn Stover Xylan-BSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M155	Corn Stover Xylan-BSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M153	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M151	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M157	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M148	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M156	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M140	Oat Xylan-BSA (covalent)	Mouse	lgG1(κ)	
Xylan-4	CCRC-M139	Oat Xylan-BSA (covalent)	Mouse	lgG1(κ)	
	CCRC-M159	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M138	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	CCRC-M147	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	LM10	Xylopentaose-BSA (covalent)	Rat	lgG2c(κ)	(McCartney et al., 2005)
	CCRC-M158	Oat Xylan-BSA (covalent)	Mouse	lgM(κ)	
	LM11	Xylopentaose-BSA (covalent)	Rat	lgM(κ)	(McCartney et al., 2005)

Table S3: Coefficients correlating the ELISA responses obtained in one experiment withthe averages of ELISA responses from six experiments.

Correlation coefficients were calculated as the dot	product of the normalized data vectors.
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mAb	Correlation Coefficient
CCRC-M30	0.923
CCRC-M56	0.986
CCRC-M16	1.000
CCRC-M2	0.998
CCRC-M1	1.000
CCRC-M31	0.998
CCRC-JIM3	1.000
CCRC-M24	0.985
CCRC-M7	1.000
CCRC-M21	0.999
CCRC-M13	0.999
CCRC-M32	0.999
CCRC-M42	0.999
CCRC-M60	0.994
CCRC-M77	0.999
CCRC-M33	1.000
CCRC-M9	0.999
CCRC-M79	0.998
JIM16	1.000
JIM1	1.000
JIM14	1.000
JIM4	0.997
JIM17	0.997
JIM8	0.997
JIM15	0.999
CCRC-M78	0.999
JIM13	0.999
JIM7	0.999
JIM136	0.998
CCRC-M34	0.987
LM7	0.991
CCRC-M38	0.999
JIM5	0.999
PAM1	0.980
CCRC-M35	1.000
CCRC-M14	0.998
CCRC-M36	0.999
JIM19	0.999
JIM12	1.000
JIM11	1.000
JIM20	1.000

LITERATURE CITED

- Stephen AM, Phillips GO, Williams PA eds, (2006) Food Polysaccharides and Their Applications. CRC Press, Boca Raton, FL, pp 1-733
- Bradley DJ, Wood EA, Larkins AP, Galfre G, Butcher GW, Brewin NJ (1988) Isolation of monoclonal antibodies reacting with peribacteroid membranes and other components of pea root nodules containing *Rhizobium leguminosarum*. Planta **173**: 149-160
- Deng C, O'Neill MA, Hahn MG, York WS (2009) Improved procedures for the selective chemical fragmentation of rhamnogalacturonans. Carbohydr Res 344: 1852-1857
- Guillon F, Tranquet O, Quillien L, Utille JP, Ortiz JJO, Saulnier L (2004) Generation of polyclonal and monoclonal antibodies against arabinoxylans and their use for immunocytochemical location of arabinoxylans in cell walls of endosperm of wheat. J Cereal Sci 40: 167-182
- Hahn MG, Darvill A, Albersheim P, Bergmann C, Cheong J-J, Koller A, Lò V-M (1992)
 Preparation and characterization of oligosaccharide elicitors of phytoalexin accumulation.
 In S Gurr, M McPherson, DJ Bowles, eds, Molecular Plant Pathology (Volume II): A
 Practical Approach, Oxford University Press, Oxford, UK, pp 103-147
- Hahn MG, Lerner DR, Fitter MS, Norman PM, Lamb CJ (1987) Characterization of monoclonal antibodies to protoplast membranes of *Nicotiana tabacum* identified by an enzyme-linked immunosorbent assay. Planta 171: 453-465
- Jia ZH, Qin Q, Darvill AG, York WS (2003) Structure of the xyloglucan produced by suspension-cultured tomato cells. Carbohydr Res **338**: 1197-1208
- Jones L, Seymour GB, Knox JP (1997) Localization of pectic galactan in tomato cell walls using a monoclonal antibody specific to $(1\rightarrow 4)$ - β -D-galactan. Plant Physiol **113**: 1405-1412
- Knox JP, Linstead PJ, King J, Cooper C, Roberts K (1990) Pectin esterification is spatially regulated both within cell walls and between developing tissues of root apices. Planta 181: 512-521
- Knox JP, Linstead PJ, Peart J, Cooper C, Roberts K (1991) Developmentally regulated epitopes of cell surface arabinogalactan proteins and their relation to root tissue pattern formation. Plant J 1: 317-326

- Knox JP, Roberts K (1989) Carbohydrate antigens and lectin receptors of the plasma membrane of carrot cells. Protoplasma 152: 123-129
- Marcus SE, Verhertbruggen Y, Herve C, Ordaz-Ortiz JJ, Farkas V, Pedersen HL, Willats WGT, Knox JP (2008) Pectic homogalacturonan masks abundant sets of xyloglucan epitopes in plant cell walls. BMC Plant Biology 8: 60-71
- McCartney L, Marcus SE, Knox JP (2005) Monoclonal antibodies to plant cell wall xylans and arabinoxylans. J Histochem Cytochem 53: 543-546
- Meikle PJ, Bonig I, Hoogenraad NJ, Clarke AE, Stone BA (1991) The location of $(1\rightarrow 3)$ - β -glucans in the walls of pollen tubes of *Nicotiana alata* using a $(1\rightarrow 3)$ - β -glucan-specific monoclonal antibody. Planta **185:** 1-8
- Norman PM, Wingate VPM, Fitter MS, Lamb CJ (1986) Monoclonal antibodies to plant plasma-membrane antigens. Planta 167: 452-459
- Peña MJ, Darvill AG, Eberhard S, York WS, O'Neill MA (2008) Moss and liverwort xyloglucans contain galacturonic acid and are structurally distinct from the xyloglucans synthesized by hornworts and vascular plants. Glycobiology 18: 891-904
- Pennell RI, Janniche L, Kjellbom P, Scofield GN, Peart JM, Roberts K (1991) Developmental regulation of a plasma membrane arabinogalactan protein epitope in oilseed rape flowers. Plant Cell 3: 1317-1326
- Perotto S, VandenBosch KA, Butcher GW, Brewin NJ (1991) Molecular composition and development of the plant glycocalyx associated with the peribacteroid membrane of pea root nodules. Development 112: 763-773
- Puhlmann J, Bucheli E, Swain MJ, Dunning N, Albersheim P, Darvill AG, Hahn MG (1994) Generation of monoclonal antibodies against plant cell wall polysaccharides. I. Characterization of a monoclonal antibody to a terminal α -(1 \rightarrow 2)-linked fucosyl-containing epitope. Plant Physiol **104**: 699-710
- Smallwood M, Beven A, Donovan N, Neill SJ, Peart J, Roberts K, Knox JP (1994) Localization of cell wall proteins in relation to the developmental anatomy of the carrot root apex. Plant J 5: 237-246
- Smallwood M, Martin H, Knox JP (1995) An epitope of rice threonine- and hydroxyprolinerich glycoprotein is common to cell wall and hydrophobic plasma-membrane glycoproteins. Planta 196: 510-522

- Smallwood M, Yates EA, Willats WGT, Martin H, Knox JP (1996) Immunochemical comparison of membrane-associated and secreted arabinogalactan-proteins in rice and carrot. Planta 198: 452-459
- Stevenson TT, McNeil M, Darvill AG, Albersheim P (1986) Structure of plant cell walls. XVIII. An analysis of the extracellular polysaccharides of suspension-cultured sycamore cells. Plant Physiol 80: 1012-1019
- VandenBosch KA, Bradley DJ, Knox JP, Perotto S, Butcher GW, Brewin NJ (1989) Common components of the infection thread matrix and the intercellular space identified by immunocytochemical analysis of pea nodules and uninfected roots. EMBO J 8: 335-342
- Wang M, Heimovaara-Dijkstra S, Van der Meulen RM, Knox JP, Neill SJ (1995) The monoclonal antibody JIM19 modulates abscisic acid action in barley aleurone protoplasts. Planta 196: 271-276
- Willats WGT, Gilmartin PM, Dalgaard Mikkelsen J, Knox JP (1999) Cell wall antibodies without immunization: generation and use of de-esterified homogalacturonan blockspecific antibodies from a naive phage display library. Plant J 18: 57-65
- Willats WGT, Marcus SE, Knox JP (1998) Generation of a monoclonal antibody specific to $(1\rightarrow 5)-\alpha$ -L-arabinan. Carbohydr Res **308**: 149-152
- Willats WGT, McCartney L, Steele-King CG, Marcus SE, Mort A, Huisman M, Van Alebeek G-J, Schols HA, Voragen AGJ, Le Goff A, Bonnin E, Thibault J-F, Knox JP (2004) A xylogalacturonan epitope is specifically associated with plant cell detachment. Planta 218: 673-681
- Willats WGT, Orfila C, Limberg G, Buchholt HC, Van Alebeek G-JWM, Voragen AGJ, Marcus SE, Christensen TMIE, Mikkelsen JD, Murray BS, Knox JP (2001)
 Modulation of the degree and pattern of methyl-esterification of pectic homogalacturonan in plant cell walls - Implications for pectin methyl esterase action, matrix properties, and cell adhesion. J Biol Chem 276: 19404-19413
- **York WS, Harvey LK, Guillen R, Albersheim P, Darvill AG** (1993) Structural analysis of tamarind seed xyloglucan oligosaccharides using β-galactosidase digestion and spectroscopic methods. Carbohydr Res **248**: 285-301
- Zablackis E, Huang J, Müller B, Darvill AG, Albersheim P (1995) Characterization of the cell wall polysaccharides of *Arabidopsis thaliana* leaves. Plant Physiol **107**: 1129-1138