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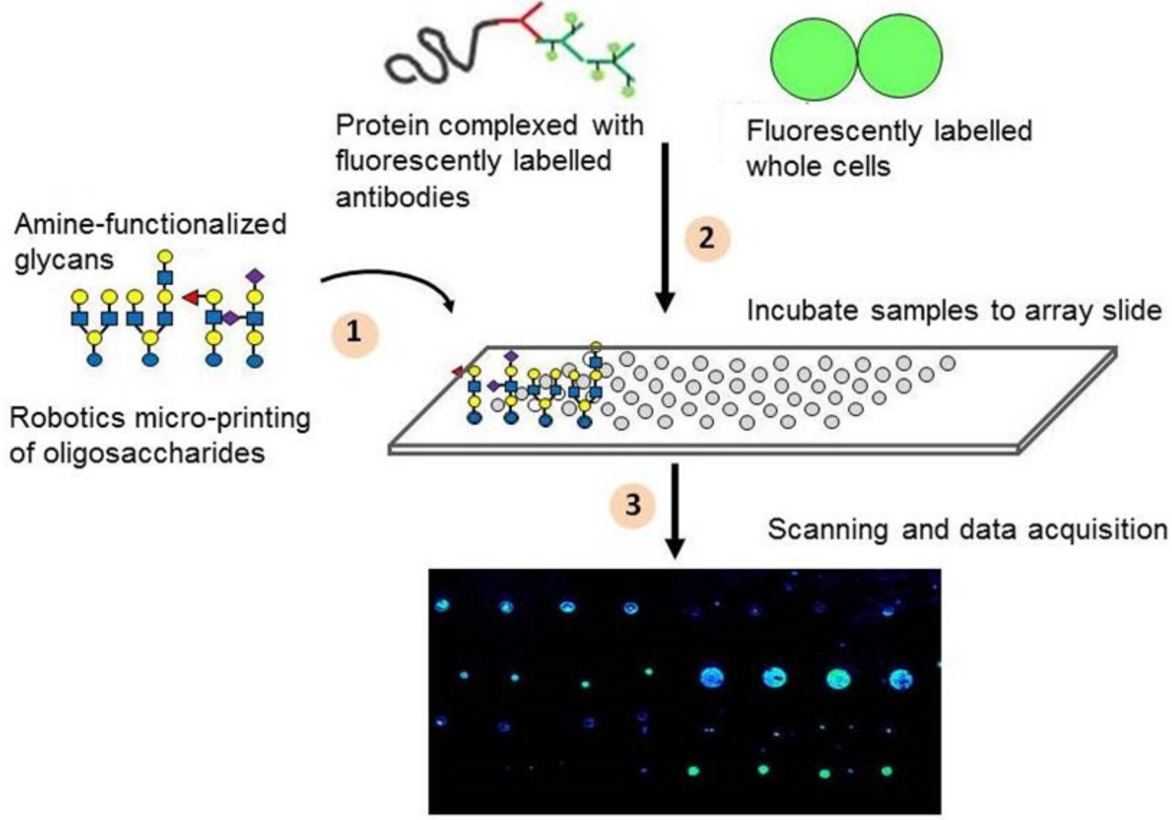
DEXTRA GLYCAN ARRAY KIT

User Manual

Overview

Glycan Array Kits - GAK001

For use in determining the glycan binding profile of proteins, eukaryotic cells, viruses, spores, bacteria and parasites.



1. Amine functionalised glycans are printed onto epoxy coated microarray slides through the use of a microarray printer.
2. Samples which can be used on the glycan array slide range from proteins to whole cells. Samples are fluorescently labelled for detection, either by fluorescent conjugate antibodies, or by directly labelling the sample with a fluorescent dye.
3. Slide images are acquired using a microarray scanner with appropriate settings for the fluorophore used. Analysis of images is performed using microarray analysis software and supplied gal file for alignment.

Please read the manual carefully prior to commencing experiment.

Kit Contents

1 x Dextra Glycan Array slide with barcode

3 x Blue Gene Frames with coverslips (volume of 65 µL).

Each slide contains three identical subarrays, with six spot replicates printed per array (See Appendix A). Fluorescent control spots are printed in each corner to aid in alignment.

Kit Storage

The Dextra Glycan Array kit should be stored unopened at 4 °C . For best results, use kit within three months of purchase.

Materials Required

- Laser scanner for fluorescent detection of glycan spots (see Appendix B for a list of recommended scanners).
    - **Check excitation/emission of dyes and scanner specifications.**
    - **Scanner specifications: 16-bit TIFF image, <10 µm resolution.**
- Array Phosphate Buffered Saline (Array PBS, Phosphate Buffered Saline with 1.8 mM $MgCl_2$, $CaCl_2$). See Appendix C. Must be prepared on the day of experiment.
- Fluorescent dye – for labelling of cells which do not express a fluorescent marker. See Appendix D for the dye preparation protocol.
- Fluorescent antibodies – for detection of protein with primary antibody. See Appendix E for list of recommended antibodies.
    - **Check Laser scanner specifications prior to selecting antibody label.**
- Primary antibody against protein of interest (if required).
- 50 mL Conical Polypropylene Centrifuge tubes.
- 50 mL 0.5% Bovine Serum Albumin in Array PBS.
- Forceps for slide handling.
- 1 mL 1% Tween 20 in Array PBS.
- Incubator (for cell based arrays).
- Centrifuge with rotor to fit 50 mL tubes.
- Petri dish or similar dish for first wash step.
- Pipettes, pipette tips and other common laboratory consumables.

Kit Contents

i) Proteins

Purified proteins are pre-complexed with antibodies in order to fluorescently label the protein, then hybridised to an array slide, washed and scanned.

Generally, recombinant proteins will have either a His- or GST- tag. In this case, the primary antibody is either a mouse anti-His or mouse anti-GST. The secondary antibody is usually a rabbit anti-mouse AlexaFluor488/555/647 and the tertiary is a goat anti-rabbit AlexaFluor488/555/647 conjugate. See Appendix E for list of recommended antibodies.

- Antibodies are added at a 4:2:1 molar ratio.
- 1-5 µg of protein is required for each array.
- Prior to incubation on the array, the protein sample must be:
  - free of glycerol
  - have <5 µM Tris-Cl
  - >80% pure for best results
- If required perform buffer exchange using either PBS or Array PBS.
- When using primary antibodies which are raised against the protein, it is recommended to perform an “antibody only control” to ensure that the antibody does not have any glycan binding properties.

Method:

1. To prepare protein samples, use 1-5 µg of purified protein and add an appropriate amount of primary, secondary and tertiary antibody to a final volume of 65 µL using Array PBS.
2. Incubate the protein and antibody complex in the dark for 10 min on ice prior to adding the sample to array slide. (Shewell *et al*, 2015)
3. Amount of antibodies will vary depending on the size of the protein of interest, i.e. 1 µg of 50 kDa protein will need 1×10^{-11} moles primary antibody, 5×10^{-12} moles secondary antibody and 2.5×10^{-12} moles tertiary antibody. If the protein has glycerol present, perform a buffer exchange prior to running the array, as glycerol causes significant background.

Sample Preparation

i) Whole Cells

For whole cell based arrays, cells must be fluorescently labelled prior to incubation on the array.

Recommended dyes are: Bodipy-SE 558 (Invitrogen D2219) or CFDA-SE (Invitrogen C1157) as described in Logan *et al*, 1998.

0.5 mL aliquots can be stored at -20 °C until required. See Appendix D for full protocol. Check Laser scanner specifications prior to choosing dye.

Method:

Note: Standard Phosphate Buffered Saline (PBS) is to be used for cell labelling. Array PBS is used only in the final step for addition of the sample to the array.

1. Log-stage cells are harvested from plate or media into PBS and washed once to remove any excess media. The cells are then labelled with 10 µM dye for 30-60 mins at 37 °C in the dark (Day *et al*, 2009).
2. To remove any free dye, the cells are centrifuged at 300-500 x *g* for 5 mins, and then washed a minimum of 3 times in PBS.
3. After labelling, adjust cell number to an OD_{600nm} of 0.2 using Array PBS. For eukaryotic cells, 1 x 10^6 CFU are added to each array. This may need to be optimised depending on cell size. If cells are prone to clumping, filter cells prior to adding them to the array and add 1% Fetal Calf Serum (FCS) or BSA to the cells (Ardnt *et al*, 2011; Wurpel *et al*, 2014).
4. Add a total volume of 65 µL of sample to each of the Gene Frames on the array. Three samples can be tested simultaneously.
5. If required, cells can be fixed in 4% paraformaldehyde (PFA) for 20 mins. Prior to incubation on the array, excess PFA needs to be removed by washing three times in Array PBS and resuspended in an appropriate volume of Array PBS.

Sample Preparation

Unpacking of slide kit

On the day of experiment, remove kit from 4 °C storage and open package to allow the slide to come to room temperature prior to commencing experiment (**Figure 1a**: Kit contents).

Handling of slide

Gloves must be worn at all times when using the Dextra Glycan Array slides. The glycans are printed on the surface of the slide, so extreme care is needed when handling the slide. The barcode area is safe to touch.

For correct orientation of the slide, the barcode should be visible.

Pre-blocking of slide

- For protein samples, pre-blocking the array slide with 0.5% BSA in Array PBS is recommended.
- Place the slide in a 50 mL tube with 0.5% BSA in Array PBS with the barcode facing up, for 5 mins.
- Remove the slide using forceps touching only the barcode. Place slide in a 50 mL tube with Array PBS and invert several times.
- Transfer the slide to an empty 50 mL tube using forceps, and dry by centrifuging for 5 mins at 200 x *g*.

Application of Gene Frames

- Set up the array slide with Gene Frame by removing the plastic protector with window and orientate Gene Frame onto slide (**Figure 1b**).
- The top of the Gene Frame will align on the top of slide and left-hand side of slide.
- Place the second Gene Frame directly under the top Gene Frame, and the third under the second Gene Frame (**Figure 1c**).
- Do not remove the top plastic protector until the sample is ready to be applied.
- Gently run finger or the forceps handle around edge of Gene Frame to ensure frame is stuck to the slide surface.

Sample Preparation

Figure 1a:
Kit contents

Figure 1b:
Positioning of the top of the Gene Frame on the Glycan Array Kit slide.
Note:
Gene Frame is placed on slide and aligns with top and left-hand edges of the glass.

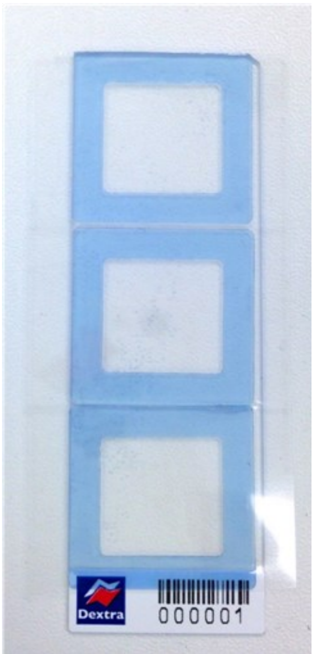


Figure 1c:
Positioning of the three Gene Frames on the Glycan Array slide.

Protocol

Incubation

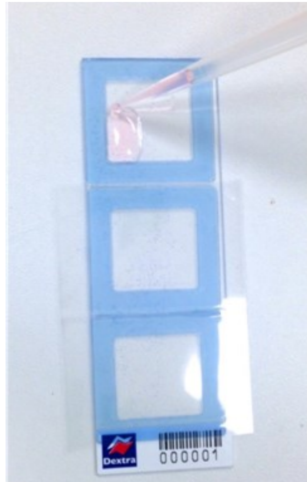
Remove the plastic protector from the first Gene Frame and pipette 65 µL of sample into the left-hand edge of the Gene Frame area, without touching the slide (**Figure 2**).

Work quickly to seal the Gene Frame using the plastic coverslip provided, avoiding air bubbles. Repeat for the second and third Gene Frames. Do not remove the plastic protector from the neighbouring Gene Frame until the plastic coverslip has sealed the previous sample to avoid cross-contamination.

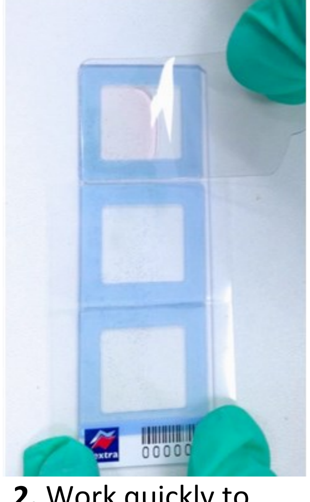
Incubation times and conditions will vary depending on sample type. The following examples are a guide:

- Proteins: 10 - 30 min at room temperature in the dark.
  Enzymes or proteins with activity, it is recommended that incubation occurs at conditions to reduce activity e.g. 4 °C or pH 8.0.
- Non-fixed cells: incubate at 37 °C for 15-30 min in the dark. Place slide in a humidified chamber (e.g. an empty tip box, with 1 cm of water in the bottom) to avoid evaporation.
- Fixed cells: 30 - 45 mins at room temperature in the dark.

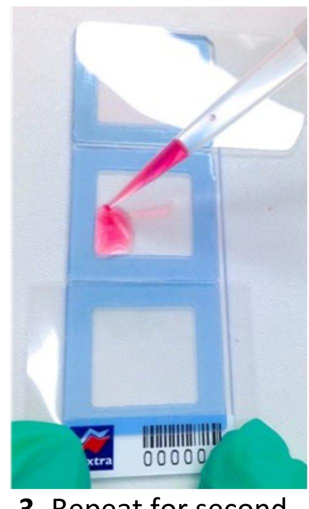
Figure 2: Application of sample to slide.



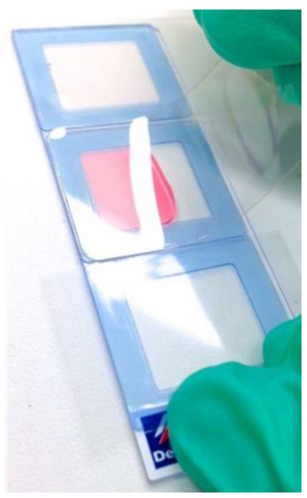
1. Remove plastic protector from Gene Frame and add sample.



2. Work quickly to seal Gene Frame with coverslip. Gently roll the coverslip on.



3. Repeat for second sample.



4. Seal sample with coverslip, avoiding air bubbles.

Protocol

Washing

After incubation, place Array PBS with 0.5% BSA (approx. 50 mL, used for preblocking slide) into a clean container (e.g. petri dish).

Submerge slide under the solution. Carefully remove coverslips and Gene Frames using forceps. Be careful not to touch the glass slide.

After Gene Frames are removed, gently wash the slide in the Array PBS with 0.5% BSA solution by slow agitation for 2 mins.

Transfer the slide to a 50 mL tube filled with Array PBS using forceps. Wash by gentle inversion for 2 mins. Repeat transfer to a new tube with new Array PBS and wash for an additional 2 mins with gentle inversion.

For whole cells, it is recommended that the slides be fixed by immersion in 4% PFA in PBS for 20 mins. Slides must be subsequently rinsed very well in Array PBS to remove excess PFA, and then dried as outlined below.

Drying and Storage

Place slide with barcode facing up into empty 50 mL tube and dry slide by centrifugation at 200 x *g* for 5 mins.

Slides must be completely dry before scanning or storage. To avoid photo-bleaching, store slides in the dark.

Scanning and image acquisition

Scan slide according to each Laser Scanner's specifications. In general, scan slides using 100% laser power with a PMT of 50-70% gain.

In the case of low signal, increase PMT gain so that control spots can be visualised. See Appendix A for slide layout.

See troubleshooting guide for additional information if required.

Data Analysis

Image analysis can be performed using most microarray analysis software (e.g. ScanArray Express, Mapix, ArrayVision or GenePix).

PLEASE NOTE: each glycan is printed in six spot replicates in each subarray.

1. Use the provided file titled “Dextra GAK001.gal” to map the subarrays on the scanned image according to Appendix A.
2. Once aligned, the data can be exported. The data is generally exported as a .csv file or .xls file for use in Microsoft Excel.

Interpretation of data

1. Use the provided statistical analysis template titled “Statistical template_Dextra GAK001.xlsx” to aid in data analysis and interpretation of results.
2. From the exported .csv or .xls data file (microarray analysis software), select and copy the 'mean-background' column data and paste it into the ‘value’ column in the statistical analysis template.
3. Any spots with a ‘fold increase’ of >1 are automatically highlighted in the statistical analysis template. These spots represent a binding interaction.

PLEASE NOTE*: the 'mean – background' data is selected for data analysis as the raw signal intensity of each spot has been normalized to the background of the slide. This data takes into account the possibility of high background noise.*
The statistical analysis template uses the background subtracted mean values of each spot. The average fluorescence intensity for each glycan is calculated and compared to the calculated fluorescence of the background. The calculated fluorescence of the background is determined by the average fluorescence of the negative control spots plus three standard deviations.
The comparison of the average spot intensity against the calculated fluorescence of the background, generates a fold increase above background, which has statistical accuracy of >99%. Only glycans with a fold increase of >1 is deemed as binding. Any spots with a fold increase of >1 are automatically highlighted in the statistical template. Please refer to instructions in the template for further guidance.

Protocol

Fee-for-service analysis is available at:
www.griffith.edu.au/science-aviation/institute-glycomics/facilities/glycan-array

Disclaimer

The Dextra Glycan Array Kit is intended for research purposes only and is not to be used for clinical diagnosis. Our products may not be resold, modified for resale, or used to manufacture commercial products without written approval by Dextra Laboratories Ltd.

Under no circumstances shall Dextra Laboratories Ltd be liable for any damages arising out of the use of the materials.

The Dextra Glycan Array Kit is guaranteed for three months from the date of purchase when handled and stored as outlined in this user's manual. In the event of any defect in product quality or merchantability, the liability of Dextra Laboratories Ltd to the buyer for any claim relating to products shall be limited to a single slide replacement where the defect can be shown to be the fault of manufacture.

References

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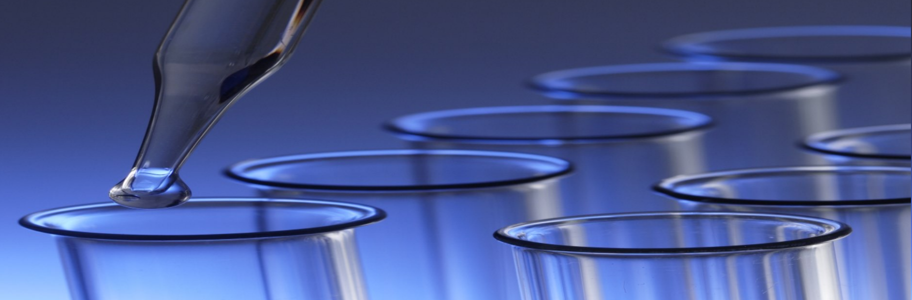
- Logan R.P., Robbins A., Turner G.A., Cockayne A., Borriello S.P., Hawkey C.J. (1998) A novel flow cytometric assay for quantitating adherence of Helicobacter pylori to gastric epithelial cells. J. Immunol. Methods 213 (1) 19-30.



Glycan Structures

Tumour antigens and oligosaccharide standards

Name	Code
Galactosyl-Tn antigen	GS203
Globotriose	L340
Tn antigen	GS103
Galβ1-6Gal	G206
GalNAcβ1-3Gal	G273
GalNAcβ1-4Gal	G274
Galα1-4Galβ1-4GlcNAc	LN340
GalNAcα1-3Galβ1-4Glc	L383

Diagram labels: GS203: -Ser; GS103: -Ser

Oligomannose core structures

Name	Code
α1-2-Mannobiose	M202
α1-3-Mannobiose	M203
α1-4-Mannobiose	M204
α1-6-Mannobiose	M206
α1-3,α1-6-Mannobiose	M336
α1-3,α1-3,α1-6-Mannopentaose	M536

Lactose based O-glycans

Name	Code
Lacto-*N*-fucopentaose I	L502
Lacto-*N*-fucopentaose II	L503
Lacto-*N*-fucopentaose III	L504
Lacto-*N*-difucohexaose I	L602
Lacto-*N*-difucohexaose II	L603
2'-Fucosyllactose	L302
3-Fucosyllactose	L303
Monofucosyllacto-*N*-hexaose III	L736
Difucosyllacto-*N*-hexaose (a)	L837
Lacto-*N*-fucopentaose VI	L514
Monofucosyllacto-*N*-hexaose I	L735

Galα1-3Gal series

Name	Code
α1-3 Galactobiose	G203
Linear B-6 trisaccharide	L330
α1-3, β1-4,α1-3 Galactotetraose	G443

Cell adhesion molecules

Name	Code
Sulpho Lewis[a]	SSN304
Sulpho Lewis[x]	SSN303
Sialyl Lewis[a] (S Lea)	SLN404
Sialyl Lewis[x] (S Lex)	SLN403

Diagram labels: SSN304: SO_3-; SSN303: SO_3-

Blood group and Lewis antigens

Name	Code
Linear B-2 trisaccharide	GN334
H-disaccaride	L205
Lewis[x]	LN303
Lewis[a]	LN304
Blood Group A trisaccharide	L305
Blood Group B trisaccharide	G323
Lewis[y]	LN423
Blood Group H Type II trisaccharide	LN320
Lewis[y] hexasaccharide (LNnDFH I)	LN607
Blood Group A tetrasaccharide	L143
Blood Group B pentasaccharide	G525
Blood Group A pentasaccharide	L505

N-Acetyllactosamine and Analogues

Name	Code
Lacto-N-Biose I	GN203
N-Acetyllactosamine	GN204
β-1-4 Galactosyl-galactose	G204
β-1-3 Galactosyl-*N*-acetyl glucosamin	GN213
Galβ1-3GalNAcβ1-4Galβ1-4Glc	GN413

Complex type N-glycans

Name	Code
Asialo galactosylated, fucosylated biantennary (NA2F)	C1026
Asialo, galactosylated, tetranatennary, N-linked glycan (NA4)	C1224
Monosialo(2,6), biantennary (A1)	SC1020
Disialo (2,6) biantennary (A2)	SC1120
Asialo, galactoysylated, biantennary (NA2)	C0920
Disialo, galactosylated, fucosylated, biantennary (A2F)	SC1127
Trisialylated, galactosylated, triantennary (A3)	SC1130
Asialo, agalacto, bisected triannary (NGA3B)	C0960

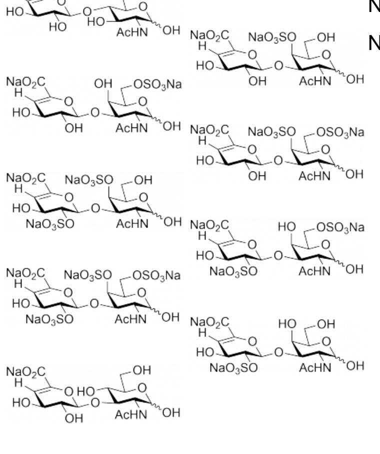
Glycan Structures

Sialylated oligosaccharides

Name	Code
Monosialyl, monofucosyllacto-*N*-neohexose	SLN807
Disialyl-TF	SLN402
3'-Sialyllactosamine	SLN302
6'-Sialyllactosamine	SLN306
LS-Tetrasaccharide a (LSTa)	SLN503
LS-Tetrasaccharide b (LSTb)	SLN516
LS-Tetrasaccharide c (LSTc)	SLN506
Disialyllacto-N-tetraose	SLN603
3'-Sialyllactose	SL302
6'-Sialyllactose	SL306

Heparin and chondroitin derived oligosaccharides
(Glycosaminoglycans from polysaccharides)

Name	Code
ΔUA→2S-GlcNS-6S	H1001
ΔUA→GlucNS-6S	H1002
ΔUA→2S-GlucNS	H1003
ΔUA→2S-GlcNAc-6S	H1005
ΔUA→GlcNAc-6S	H1006
ΔUA→2S-GlcNAc	H1007
ΔUA→GlcNAc	H1008
ΔUA→GalNAc-4S (Delta Di-4S)	C3202
ΔUA→GalNAc-6S (Delta Di-6S)	C3203
ΔUA→GalNAc-4S,6S (Delta Di-disE)	C3204
ΔUA→2S-GalNAc-4S (Delta Di-disB)	C3205
ΔUA→2S-GalNAc-6S (Delta Di-disD)	C3206
ΔUA→2S-GalNAc-4S-6S (Delta Di-triS)	C3207
ΔUA→2S-GalNAc-6S (Delta Di-UA2S)	C3208
ΔUA→GlcNAc (Delta Di-HA)	C3209
Heparin	PS115
Chondroitin sulfate	PS109
Heparin unsaturated disaccharide I-H	H1009
Heparin unsaturated disaccharide II-H	H1010
Heparin unsaturated disaccharide III-H	H1011
Heparin unsaturated disaccharide IV-H	H1012



N-Acetylgalactosamine core structures

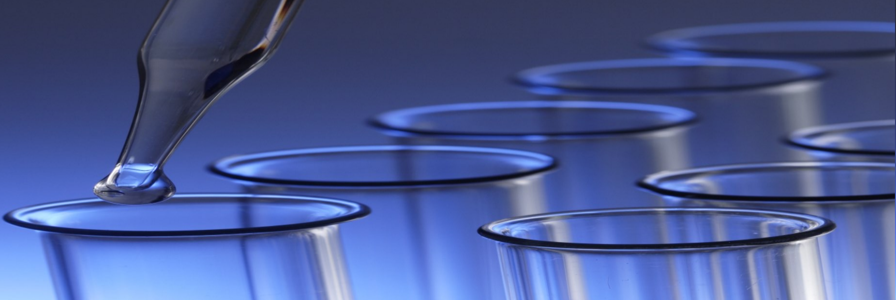
Name	Code
β-1-3 Galactosyl-*N*-acetyl galactosamine	GN206
N,*N*'-Diacetyl chitobiose	C8002
N,*N*',*N*''-Triacetyl chitotriose	C8003
N,*N*',*N*'',*N*'''-Tetraacetyl chitotetraose	C8004
N,*N*',*N*'',*N*''',*N*'''',*N*'''''-Hexaacetyl chitohexaose	C8006
N,*N*',*N*'',*N*''',*N*''''-Pentacetyl chitopentaose	C8005
β1-2 *N*-Acetylglucosamine-mannose	M292
Bianntennary *N*-linked core pentasaccharide	M592

Maltooligosaccharides

Name	Code
Maltotriose	G302
Maltotetraose	G402

Neutral and sulfated Galacto-oligosaccharides

Name	Code
Neocarratetraose-$4^{1,3}$-di-O-sulphate (Na+)	C1003
Neocarratetraose-4^{1}-O-sulphate (Na+)	C1004
Neocarrahexaose-$2^{4,41,3,5}$-tetra-O-sulphate (Na+)	C1006
Neocarrahexaose-$4^{1,3,5}$-tri-O-sulphate (Na+)	C1010
Neocarraoctaose-$4^{1,3,5,7}$-tetra-O-sulphate (Na+)	C1011
Neocarradecaose-$4^{1,3,5,7,9}$-penta-O-sulphate (Na+)	C1012



Troubleshooting

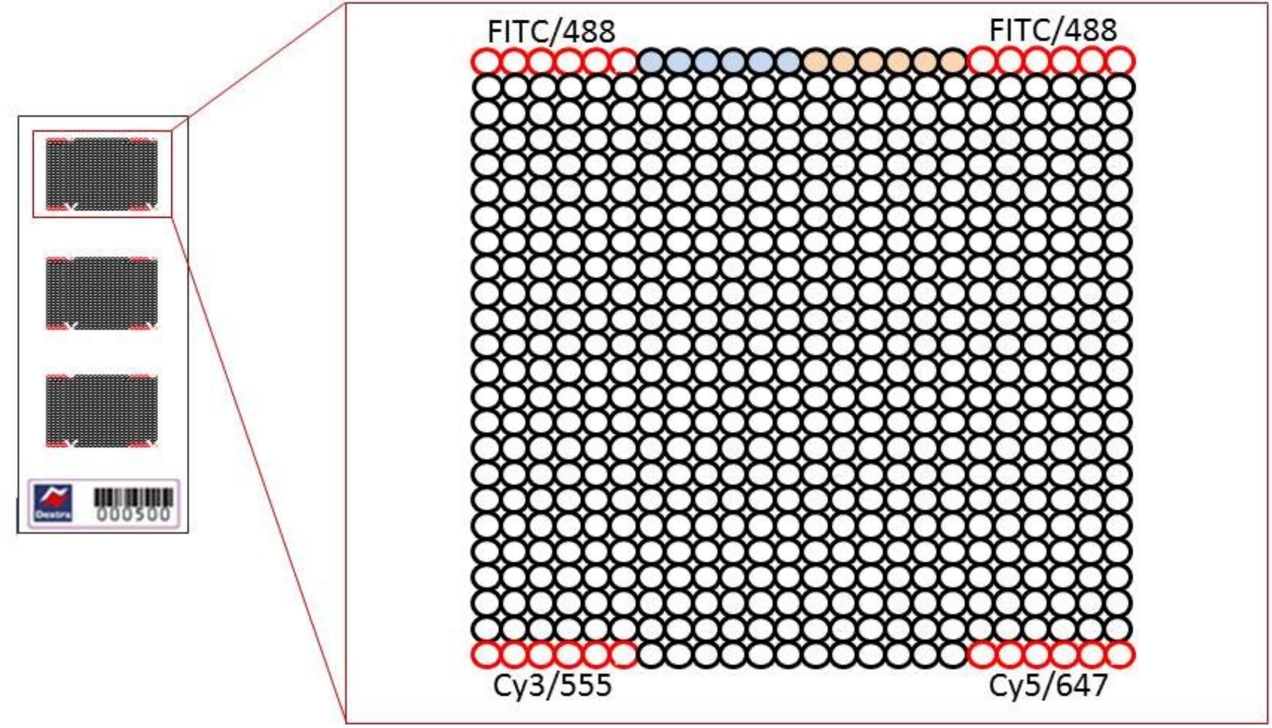
Problem	Cause	Recommendation
High background	Insufficient washing	Perform additional 2 min wash with Array PBS or Array PBS with 0.001% Tween 20.
	Areas of intense signal (blobs) where sample was placed	Place coverslip over Gene Frame quickly after loading sample. Sample drying onto slide causes uneven signals.
	Uneven signals	Slide not submerged under buffer when coverslip removed. Sample starts to dry onto slide.
	Cells clumping on slide	Add 1% BSA or FCS and filter to remove cell clumps prior to addition to slide.
	Fluorescence due to PFA	PFA auto-fluoresces. Ensure all PFA is removed by adequate washing prior to scanning.
Antibody controls show binding	Some antibodies have glycan binding properties.	Use alternative antibodies.
Low or weak signal	Primary antibody blocks glycan binding site of protein	Add protein, then antibody complex sequentially. Incubate for 30 mins, wash in Array PBS then antibody complex. Incubate on slide for an additional 30 min.
	Inadequate detection	Increase PMT gain.
	Low protein present	Increase amount of protein added to array and check that preparation conditions are not causing protein degradation.
	“Black Spots” seen on array	Reduce agitation when washing. Wash slides with very gentle inversion, once every minute only.
	Check extinction coefficient of fluorescent antibody/dye	Signal may need to be amplified with addition of secondary/tertiary fluorescent antibody.
	No spots are seen	No glycan binding properties or sample has glycosidase activity. Reduce temperature and incubation time in the case of glycosidase activity.

Troubleshooting

Problem	Cause	Recommendation
Uneven signal	Bubbles formed during incubation or sample evaporation.	Place slide in humidified chamber (a plastic box such as a pipette tip box with 1 cm water in bottom may suffice. Ensure the slide is flat and elevated above the water, not submerged).
	Bubbles formed when coverslip is placed on Gene Frame	Gently manoeuvre bubbles to outer edges of Gene Frame. Do not remove Gene Frame.
Scratches, dust or swirls	Touching of slide by pipette tip or when handling slide	Ensure pipette tip does not touch slide surface when loading sample. Take extreme care when handling slides. Use forceps where possible.
	Dust present on slide	Filter sample prior to loading and work in dust-free environment.
	Spotty slides	Filter all solutions prior to use. Array PBS can precipitate and must be made fresh on day of experiment.
	Swirls and whirls	Presence of high amounts of glycerol in sample. Buffer exchange to remove glycerol.

Appendix A

Slide Layout



- Fluorescent control spots
- Printed glycans
- Layout of 6 spot replicates

Appendix B

Scanner Specifications:

Standard Glass Slide:	1" x 3" (25 mm x 75 mm) microscope slides
Thickness:	1 mm
Light and Detector Orientation:	Facing array
Scanned Area:	22 mm x 73 mm
Focus:	Auto focus or adjustable (+/- 200 µm)
Excitation:	FITC (Blue) Channel 488 nm
	Cy3 (Green) Channel 532 nm
	Cy5 (Red) Channel 633 nm
Resolution:	10 µm
Dynamic Range:	>3 orders of magnitude
Detection Output:	16-bit TIFF

Recommended Scanners:

- InnoScan 710/900/1100 Microarray Scanner (Innopsys)
- GenePix® 4000A/B (Molecular Devices)
- GenePix® 4100A (Molecular Devices)
- GenePix® Professional 4200A (Molecular Devices)
- ScanArray® Express (PerkinElmer)
- ScanArray® Express HT (PerkinElmer)
- ScanArray® 4000 (PerkinElmer)
- ScanArray® 4000XL (PerkinElmer)
- ScanArray® 5000 (PerkinElmer)
- ScanArray® 5000XL (PerkinElmer)
- LS Series Laser Scanner (Tecan Group AG)
- AlphaScan Microarray Scanner (Alpha Innotech)
- aQuire 110 V (Genetix)
- aQuire 240 V (Genetix)
- VersArray ChipReader 5 µm System (Bio-Rad)
- VersArray ChipReader 3 µm System (Bio-Rad)

Note: This is not the full list of microarray scanners available. Please check scanner specifications in relation to excitation/emission range, slide size and scanning resolution. Scanning resolution >10 µm may result in low signal intensities.

Appendix C

Array Phosphate Buffered Saline (Array PBS):

Making 1 x Array PBS:

To 360 mL of milliQ water add 20 mL of stock solution A (348 mM Na_2HPO_4, 70 mM NaH_2PO_4; 20 x concentration) and then add 20 mL of stock solution B (18 mM $CaCl_2$, 70 mM KCl, 18 mM $MgCl_2$, 2740 mM NaCl; 20 x concentration). Adjust pH to 7.3 and sterilise by passing solution through a 0.22 µm filter.

The final solution will have CaCl2 and MgCl2 at 1.8 mM. The 1 x Array PBS must be prepared daily as crystals will form over time. Stock solutions can be made and stored without issues.

Appendix D

Preparation of fluorescent dyes:

Prepare Bodipy-SE 558 (Invitrogen D2219) or CFDA-SE (Invitrogen C1157) as outlined in Logan *et al*, 1998).

Briefly, prepare dye stock to 40 mM in anhydrous DMSO. Aliquot dye into 40 µL batches and store at -20 °C in the dark until required. To make up, resuspend this into 1 mL of 100% ethanol and add to 19 mL of standard PBS. Aliquot diluted dye into 250 µL lots and store at -20 °C, and keep in the dark. The concentration is 20 µM. For labelling of cells, use 100 µL of cell suspension and add 100 µL of 20 µM dye and incubate at 37 °C (or growth temperature) for 30-60 mins. Centrifuge and wash in PBS twice before adjusting to correct OD.

Appendix E

Recommended products:

Dyes:

5(6)-CFDA, SE; CFSE (5-(and-6)-Carboxyfluorescein Diacetate, Succinimidyl Ester), mixed isomers (Molecular Probes, Life Technologies) Catalogue number C1157

BODIPY® 558/568 NHS Ester (Succinimidyl Ester) (Molecular Probes, Life Technologies) Catalogue number D2219

Antibodies:

His-Tag (27E8) Mouse mAb #2366 (Cell Signalling) Catalogue number 2366S or 2366P

Rabbit anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate (Molecular Probes, Life Technologies) Catalogue number A11059

Rabbit anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 555 conjugate (Molecular Probes, Life Technologies) Catalogue number A21427

Rabbit anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 647 conjugate (Molecular Probes, Life Technologies) Catalogue number A21239

Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate Molecular Probes, Life Technologies) Catalogue number A11034

Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 555 conjugate (Molecular Probes, Life Technologies) Catalogue number A21429

Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 647 conjugate (Molecular Probes, Life Technologies) Catalogue number A21245

This manual is also available for download from www.dextrauk.com/glycanarraymanual

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