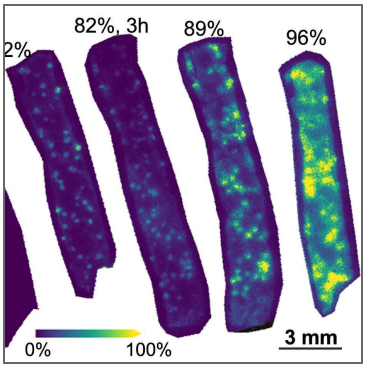

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Spatial N-glycomics with MALDI-MSI for human kidney tissue V.3

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DISCLAIMER

Protocol is adapted from "MALDI Imaging Mass Spectrometry of N-glycans and Tryptic Peptides from the Same Formalin-Fixed, Paraffin-Embedded Tissue Section" Angel, P. M.;Mehta, A.;Norris-Caneda, K.;Drake, R. R. *Methods Mol. Biol.* **2017**,*1788*, 225–241, DOI: 10.1007/7651_2017_81

Protocol utilized in "Controlled Humidity Levels for Fine Spatial Detail Information in Enzyme-Assisted N-Glycan MALDI MSI" Veličković, D.; Sharma, K.; Alexandrov, T.; Hodgin, J.B.; Anderton, C.R *J. Am. Soc. Mass Spectrom.* **2022**, 33, 1577−1580, DOI: 10.1021/jasms.2c00120

ABSTRACT

This protocol describes the procedure to obtain high quality MALDI mass spectrometry images of N-linked glycans from formalin-fixed paraffin embedded tissue. This protocol is optimized for human kidney biopsy tissue as part of the Kidney Precision Medicine Project.

Protocol status: Working
We use this protocol and it's working

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Scope

1 This protocol describes the procedure to obtain high quality MALDI mass spectrometry images of N-linked glycans from formalin-fixed paraffin embedded tissue.

Health and Safety

2 Wear nitrile gloves and safety glasses. Follow standard laboratory safety procedures.

Equipment

3 Equipment Required:

3.1 8 Coplin jars

3.2

5 slides mailer with side opening

3.3 pH strips (optimized for acidic pH)

3.4 Vegetable steamer (antigen retrieval device): we use AROMA 8-Cup Cool-Touch Rice Cooker

3.5 Flatbed color scanner (we used EPSON PERFECTION V500 PHOTO)

3.6 Zeiss 710 laser scanning confocal (LSC) microscope equipped with a 20X EC Plan NEOFLUAR objective

3.7 Syringe pump capable of 25 µl/min

3.8 Pump capable of 100 µl/min

3.9 TM-Sprayer (HTXimaging)

3.10 Home-designed chamber for incubation:
Note: This consists of a rubber gasket sealed glass container (the jar with lid, KORKEN, IKEA of Sweden. Diameter 11 cm; high 10.5 cm, volume 0.5 L) in which a 50 ml glass beaker and a set of weights is placed.The weights are required to keep the glass beaker from floating.

Chemicals & Enzymes

4 Chemicals & Enzymes:

4.1 Xylenes

4.2 200 proof ethanol

4.3 Water

4.4 Citraconic anhydrous buffer

4.5 KNO_3

4.6 1 M HCl

4.7 PNGase F enzyme (PRIME, 50 U/µg (lyophilized))

Procedure

12h 30m

5 Formalin fixed tissue should be sectioned at 7 µm and mounted on indium-tin-oxide (ITO) glass slides (25 mm x 75 mm). 30m

5.1 Slides should be dried overnight at 37^{o}C (in slide mailer) 12h

Antigen Retrieval Prep

10m

6 Prepare Citraconic buffer: 10m

6.1 25 ml distilled water or HPLC grade water into a 50 ml falcon tube

6.2 Add 25 µl of citraconic buffer to the water

6.3 Add 24 µl of 1 M HCl

6.4 Agitate tune after capping.

6.5 Add water to a total of 50 ml

6.6 Agitate tube to mix

6.7 Check that pH is around 3.0 ± 0.5 by spotting 2 µl of the prepared buffer onto a pH strip

Dewax Slides

1h 5m

6.8 Heat slides at 60°C for one hour. 1h 5m

6.9 Remove and cool to room temperature, usually 5 minutes.

Dewax Washes

21m

6.10 Use the coupling jars for dewaxing and washing tissues (by submerging slide mounted tissues 21m

6.11 Xylenes 3 minutes, repeating a total of two times.

6.12 100% ethanol 1 minute, repeating a total of two times.

6.13 95% ethanol 1 minute

6.14 70% ethanol 1 minute

6.15 Distilled water 3 minutes, repeating a total of two times.

6.16 Dry slides in desiccator 5 minutes

6.17 Scan each slide, minus the surrounding sample holder, at a minimum of 1200 ppi resolution using flatbed scanner. This will be needed for image registration in FlexImaging during imaging acquisition. Samples for higher resolution will require a higher resolution scanned image. For example, images acquired with a ≤ 50 µm step size require a 2400 dpi scanned image.

Obtaining high-resolution (5x-20x) bright field images using PALM Microb…

6.18 Turn on power supply, and key switch on PALM control unit.

6.19 Mount the slide in the slide holder and place it in the microscope

6.20 Run the PALMRobo software

6.21 In “View” Tab find "Navigation Window".

6.22 In the small screen display at the microscope, select 10x Objective and adjust focusing by turning the knob on the microscope.

6.23 In the "Navigation window" find top left corner of the tissue and select: "Set ROI top left. Next, find bottom right corner of the tissue and select "Set ROI bottom right"

6.24 Click "Scan"

6.25 Save tile images after scanning is done.

Antigen Retrieval Prep

7 Heat slides in vegetable steamer:

7.1 Preheat the vegetable steamer to generate steam by pressing the “cook” switch prior to retriev 15m
procedure (example, preheating takes ~15 min)

7.2 Add ~ 10 ml of the buffer to a 5-slide mailer with side opening

7.3 Place no more than 3 slides per 5-slide mailer with side opening. Slides should be placed with tissue facing outward to the solution in positions 1 and 5, NOT facing the slide mailer walls. Position 3 may face either way

7.4 Completely fill the slide mailer the rest of the way with buffer

7.5 If the mailer has no holes punched in the lid, only snap close one corner of the mailer

7.6 Place the mailer in the corner of the vegetable steamer

7.7 Maintain “cook” option for 30 minutes 30m

8 Cool the slides after antigen retrieval:

8.1 Remove mailer and place in a tub with cool water from the faucet. Water should not go over the top of the mailer

8.2 Allow to cool for 5 minutes 5m

8.3 Remove half the buffer from the mailer and replace with distilled water.

8.4 Allow to cool 5 minutes on countertop 5m

8.5 Repeat removal of half the buffer two more times, each time with 5 minutes of cooling 5m

8.6 Complete by rinsing in 100% distilled water

8.7 Dry the slides 5 minutes in the desiccator 5m

8.8 Check to ensure scanning of the slides has been performed

8.9 For scanning, scan one slide each at 1200 dpi

Application of PNGase F Solution

9 Prepare PNGase F solution:

9.1 Prepare 0.1 µg/µl PNGase F in water: resuspend lyophilized enzyme in 1 mL water

9.2 Ensure that enough solution is prepared, e.g. three full slides takes approximately 1 ml of solution, spraying at 25 µl/min

10 Spray the PNGase F solution:

10.1 Using the syringe dedicated to PNGase F enzyme solution, rinse the syringe with water by screwing in the needle tip, filling with 3 or more ml of water, and aspirating into waste

10.2 Fill with PNGase F solution ensuring that there are no bubbles in syringe. Tip: After loading all the PNGase F solution required, pull a small volume of air into the syringe. Gently dispense the syringe until the large air bubble is gone.

10.3 Remove the needle tip and fasten the syringe to the TM-Sprayer line used for PNGaseF. Place the syringe onto the red syringe pump. Check that the syringe head is snug against the dispense head of the syringe pump. Ensure that the diameter is set appropriate to the syringe and the rate is set at 25 µl/min. Do not start the pump at this time.

10.4 Place the samples in the TM-Sprayer tray, fastening them with tape.

10.5 Set up TM-Sprayer, referring to the guide for the TM-Sprayer. Temperature should be set to 45°C with 15 passes, velocity of 1200, and 3 mm offset. Set temp of the stage to be 40°C

10.6 Pressure reading on the front of the TM-Sprayer should be 10 psi.

10.7 Start the syringe pump.

10.8 Use a dummy slide to check the TM-Sprayer nozzle for spraying of solution. It generally takes about 1-3 minutes (100 µL) to start spraying.

10.9 Once moisture is detected on the dummy slide, press Start on the TM-Sprayer. PNGase F solution will be applied in a thin layer onto target tissue.

11 Incubation PNGase F digest:
To prevent liquid from evaporating too fast and the enzyme from becoming inactive, a wet atmosphere is maintained by placing the ITO slide into a sealed incubation chamber filled with 150 ml saturated KNO_3 solution and pre-incubated at 37.5 °C.

11.1 After application of PNGase F onto the slide, place it on top of a 50 ml glass beaker in the incubation chamber.

11.2 Incubate 2 hours at 37.5 ^{o}C 2h

11.3 After incubation, remove the slide from the incubation chamber and let dry in the desiccator (1 15m min).

11.4 Store the slide in a 5-slide mailer to protect the released glycans. If matrix cannot be sprayed the same day, store at -20°C. It is preferred to immediately spray matrix onto the slide.

Application of Matrix

12 Prepare the CHCA Matrix:

12.1 Prepare CHCA matrix at 7 mg/ml in 50% acetonitrile/0.1% TFA.Add 0.042 g CHCA to 6 ml 50% acetonitrile/0.1% TFA. Prepare fresh each time in a 15 ml falcon tube.

12.2 Vortex briefly and sonicate 5 minutes. 5m

12.3 Small chunks may remain in the bottom of the falcon tube. Make sure that there are not loaded into the TM-Sprayer loop as they will clog components of the TM-Sprayer.

12.4 Filter CHCA solution using Millex (Millipore) 0.2 µm syringe filter.

13 Spray the CHCA Matrix:

13.1 Fill the glass-5ml syringe with CHCA solution ensuring that there are no bubbles in syringe. Tip: After loading all the solution required, pull a small volume of air into the syringe. Gently dispense the syringe until the large air bubble is gone.

13.2 Remove the needle tip and fasten the syringe to the TM-Sprayer line going to the 6-port valve.

13.3 Move the switch to “LOAD” and steadily depress the syringe until all the sample is loaded. Note: Do not load air bubbles or undissolved matrix.

13.4 Make sure that the pump is flowing at 0.1 ml/minute. Pump pressure should be 30-40 psi when flowing at 0.1 ml/min.

13.5 Place the sample in the TM-Sprayer tray, fasten them with tape.

13.6 Set up TM-Sprayer referring to the guide for the TM-Sprayer. Temperature should be set to 80°C with 10 passes, velocity of 1300, and 2.5 mm offset.

13.7 Pressure reading on the front of the TM-Sprayer should be 10 psi.

13.8 Move the 6-port valve switch to “Spray”.

13.9 Use a dummy slide to check the TM-Sprayer nozzle for spraying of solution. It generally takes about one minute to start spraying matrix.

13.10 Once matrix is detected as an opaque solution on the dummy slide, press Start on the TM-Sprayer. CHCA solution will be applied in a thin layer onto target tissue.

13.11 When finished, matrix coated slides may be imaged immediately or stored in a desiccator.

MALDI Imaging MS Acquisition

14 Put the slide in the MALDI holder and load it in the 15 T FTICR- instrument

15 Load the method for N-glycan analysis Parameters of the method: m/z range 800-4,000; R=512k, laser power=28-32%, laser focus=minimum, laser shots=200, frequency=2,000

16 Teach plate

17 Select measurement region in FlexImaging

18 Run acquisition through FlexImaging

MALDI Imaging Analysis

19 Open SCiLS Lab

19.1 Load data to SCILS lab

19.2 Convert data to imZML using complete spectra

20 Upload imZML files to METASPACE

20.1 Under Annotation settings select: “NGlycDB-v1” for Metabolite database

20.2 Under Annotation settings select: “+Na” as Adducts