Jul 17, 2024

BAF_S03_Shimadzu MALDI 8030

DOI
dx.doi.org/10.17504/protocols.io.kxygxywo4l8j/v1

Nicholas Sherman[1]

[1]University of Virginia Biomolecular Analysis Facility Core

Biomolecular Analysis Fac…

Taylor Pierce
UVA

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DOI: dx.doi.org/10.17504/protocols.io.kxygxywo4l8j/v1

Protocol Citation: Nicholas Sherman 2024. BAF_S03_Shimadzu MALDI 8030. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygxywo4l8j/v1

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

Created: June 12, 2024

Last Modified: July 17, 2024

Protocol Integer ID: 101680

Keywords: MALDI, TOF, open-access

Abstract

General process for operation and data acquisition for the open access MALDI-TOF.

Materials

DHB Single use matrix - Thermo, PI90033
CHCA Single use matrix - Thermo, PI90031
SA Single use matrix - Thermo, PI90032
TOFMix Kit - Reagents, biotech, MALDI matrix and calibrants kit, Shimadzu, TO-724R00
ACN - Fisher chemical A955-4, Acetonitrile, optima LC/MS
Water - Fisher Chemical, W6-4, Optima LC/MS
TFA - Pierce™, Trifluoroacetic Acid, part number: 28903.
Pipette tips - Fisher Brand, yellow, part number: 02-681-151
Glass Amber vials - Thermo, 6PK1655
MALDI Target - 1x48 2.8mm wells [431R00]
Shimadzu 8030 MALDI

Safety warnings

Always use gloves when handling the target plates
Always ensure the sample is completely dry before inserting into the equipment, a wet target plate will damage the instrument

Before start

To prepare 10 mL of the solution (70% ACN, 0.1% TFA) needed for matrices:
- obtain a 20 mL glass bottle
- add 7 mL 100% ACN, 3 mL H20, and 5 uL 100% TFA

To prepare the Calibrant:
- obtain a 1.5mL amber glass vial
- obtain a 1 uL aliquots of the TofMix (aliquots should be prepared and stored a 0.5mL eppendorf provided with the TofMix kit -- refer to data sheet)
- add 74 uL 70% ACN 0.1% TFA solution to the Eppendorf and mix well
- add that mixture to the labeled amber vial

To prepare the Matrices: (CHCA, SA, and DHB)
- obtain a 1.5 mL amber glass vial and label it with the matrix name that you will be preparing
- obtain one aliquot of the matrix from the single-use kit.
- add 100 uL of 70% ACN 0.1 %TFA to matrix Eppendorf and use the pipette to mix well
- once mixed, and all powder is dissolved, add to the amber vial

This is the same for all 3 matrices

Prepare samples:

1 At our facility, CHCA matrix and TOFMix calibrants are prepared weekly and are ready to use, diluted in 70%ACN with 0.1% TFA. Refer to the manufacturer´s data sheets for matrix and TOFMix preparation procedures.
To choose a proper matrix and dilution of a specific sample, refer to the training material available or to specific scientific papers for your sample type.
Here, we described shortly to prepare and spot peptide calibrants present in the TOFMix mixture.

1.1 Get a 0.6 mL new tube: pipette 1 uL of TOFMix --> add 1 uL of CHCA matrix --> mix by up and down movement --> spot 1 uL of the mixture on one of the small spots for calibrants at the MALDI target.
Prepare and spot your samples. Let it dry completely to be ready to load the target in the MALDI instrument.

Prepare for acquistion:

2 Sign into the 'MALDI 8030' account on the computer using a set password

3 You will need the "MALDI Solutions Data Acquisition" software and the 'current instrument status' app.

4 Double-click on "current instrument status". On the pop-up window that follows, select 'use the one on my local computer', click on the connect button, and a small window will open showing the instrument status so you can refer back to it to check the vacuum pressures

5 Double-click on "data acquisition software". On the pop-up window that follows, select 'use the one on my local computer', click on the next button and login with username and password, then click finish and the software will open.

6 Software will open directly at acquire Tab. This is the main tab for data acquisition and to set parameters.
Load a saved standardized acquisition method that matches best with your analysis or set specific parameters. On our system do as follows:

7 **Acquisition software --> Acquire Tab:**
Click LOAD --> Click the plus sign on the left of CREATOR --> Click the plus sign on the left of OPERATOR --> Click the plus sign on the left of MATCHES SO FAR --> Select Low_mass_Acquisition (for mass range: 300 - 4000 m/z - use this for TOFMix calibrants) or Select High_mass_Acquisition_BSA66 kDa (for mass range: 40000 - 80000 m/z) or Select Cit c

for mass range: 5000-20000 m/z) --> Click the OPEN button to apply the chosen acquisition parameters

8 **Acquisition software --> Process Tab:**
Any parameter set in this tab will be automatically applied to the next acquisition, you can always change processing parameters and apply to any acquired data. You can load saved standardized processing that matches best with your analysis or set specific parameters before you start acquisition. On our system do as follows:
Click LOAD --> Click the plus sign on the left of CREATOR --> Click the plus sign on the left of OPERATOR --> Select No processing - we use this for most of analysis of small molecules and peptides, Low_mass_Acquisition is Shimadzu's suggested processing parameters for mass range: 300 - 4000 m/z or Select High_mass_Acquisition_BSA66kDa (for mass range: 10000 - 80000 m/z)

9 **Acquisition software --> Target Tab:**
Make sure that the "1x48 2.8mm wells [431R00]" target is selected, if it is not do as follows:
Click on the target arrow symbol --> click on the plus sign in front of 431R00 --> Select the 1x48 2.8mm wells and click on Open.

9.1 This instrument is capable of acquiring data on Positive or Negative mode. If you are running on Positive mode, ensure "Linear" is selected in the Tune file. If running on Negative mode, "Linear Negative" should be selected. To select the correct tune:
Click on the arrow near to TUNE --> click POLARITY --> POSITIVE --> MATCHES SO FAR --> Linear --> OPEN or
Click on the arrow near to TUNE --> click POLARITY --> NEGATIVE--> MATCHES SO FAR --> Linear negative --> OPEN

Acquiring data --> acquire tab

10 At the acquire tab, start loading the target in the instrument:
Click on Open Door --> wait until the green light flashes on the front of the instrument --> open the door and USING CLEAN GLOVES, insert the target plate, then close the door --> a small pop-up window will appear with the message: Door closed, confirm a plate has been loaded --> click CONFIRM --> Click on the Current Instrument Status - you will be able to see the Analyzer pressure reaching ~4-5 E-6 mbar and turning to green, it will take 2-3 min for the instrument to stabilize.

11 To define the position of the spot: click on Spots, click on the arrow at Manual Positioning (Rastered) --> Down in the the go to: digit the spot location. First go to the calibrant spot, for example, x1. --> click on the HUMAN icon - the target wil move to selected spot and you can see in the camera image (top right corner) --> click anywhere out of this window --> Since you have already loaded the acquisition method, all other parameters are already loaded and you can start acquiring.

11.1 Click the operate button - voltages will be turned on, you can check pressures and voltages opening the minimized instrument status window. Change laser power to 30 for calibrants (for a distinct type and molecular mass of molecules, adjust the laser power for optimization - the range is 0 to 180 --> refer to User Guide)--> Click FIRE button to start acquiring data.

11.2 You will see a real-time acquisition. By default, Unprocessed data will appear at the top and the processed data at the bottom. During acquisition, you can: clear the data, pause & resume or stop acquisition.

11.3 Once the acquisition is finished, you can use the left menu to show/hide spectrum: profile, average, processed, peaks.

Calibrate the instrument:

12 After TOFMix data acquisition --> Select the Calibrate tab --> Click on LOAD REFERENCES --> Click on the plus signal in the left side of CREATOR --> Click on the plus signal in the left side of OPERATOR --> Click on TOFMIX --> Click OPEN --> Uncheck the Apply non-linear correction --> Set the tolerance to 5 Da --> Press the calibrate button and a green sign should show up --> repeat this process decreasing the tolerance by 1 Da at a time, then go down to 900 mDa and continue same procedure decreasing the tolerance by 100 mDa until reaching 500 mDa with a green sign.
- if the calibrate button turns green, the calibration is successful. Once you got down to 500 mDa and a successful calibration. Save calibration: save the calibration and add a name including the calibrant used, pulse extract and the laser power applied. i.e. --> file name "TOFMix PE 2465 LP30". Select your lab folder at Project and Date as Batch name.
- if the calibrate button turns amber or red, the calibration fails. So, increase the Tolerance value (5-10
Da), press calibrate or re-acquire data to get a better spectrum.

Save acquired spectrum

13 Click on the save icon at the spectrum window.
Acquisition name: any that you want
Project: add your Lab Name - Ex: Sherman Lab
Batch ID: the date you are acquiring
Click on save button – this data you can open at any time in the software and make any change on the processing.
If you want to save a picture of the spectrum, at top left corner of the spectrum, click on COPY, COPY display and paste it to ppt.

Export mass list

14 On the right, look for "display type" and select MASS LIST --> Click on EXPORT MASS LIST --> Click on ICON --> Select C:, Mass List Export Folder and in the file name start with your lab underscore your sample name/ID --> click OK

Remove the target and finish analysis

15 In the Acquire tab, click on OPEN DOOR --> Remove the target using clean gloves --> Properly store the target --> Close the door and confirm it closed --> Confirm that the instrument is on Standby (Cyan light in the front panel and in the software: Vacuum State: Standby --> Close the software: Do you want to quit the Data Acquisition? Yes. Then, In the Windows icon, click on user, click on lock.