PROTOCOL

truXTRAC™ MALDI-TOF MS Protocol

Heat-inactivated Preparation Protocol for
Mycobacteria Colony Samples and
Analysis on the Bruker MALDI Biotyper®
MALDI-TOF Mass Spectrometer

Part Number: 010324 B
Date: February 16, 2016

REVISION HISTORY

Part Number	Revision	Date	Description of change
010324	B	Feb 16, 2016	Eppendorf tube and access microTUBEs
010324	A	Feb 1, 2016	Initial release

INTENDED USE

This protocol is for Research Use Only. For general instructions and maintenance of the M220 instrument, computer, and software, refer to Covaris document 010157 M220 Manual Rev E.

This protocol is for the sample preparation of heat-inactivated mycobacteria cultured on agar plates or tube-slants for MALDI-TOF MS identification with the Bruker MALDI Biotyper® mass spectrometer.

Colonies should be clearly visible (for example, not original inoculum). Collect bacterial colonies using a sterile, disposable 1 µl inoculation loop (approximately 4-8 mg of cells). This Adaptive Focused Acoustics® (AFA™) sample preparation protocol includes a heat inactivation step prior to AFA processing, using Eppendorf PCR tube #951010006. The heat inactivation conditions of time and temperature (100°C for 30 minutes) are identical to those of the Bruker MycoEx™ sample preparation protocol. The Bruker MALDI Biotyper database is based on samples that had this initial heat inactivation conditions, and deviation from these initial microbe inactivation conditions may adversely impact identification of microorganism.

This protocol uses the microTUBE-130 Glass Beads Pre-Slit Screw-Cap, enabling automated or mechanical pipetting.

SUMMARY OF AFA OPERATING CONDITIONS AND SUPPLIES

	Mycobacteria From Culture
AFA Instrument	M220
Peak Incident Power	40 Watts
Duty Factor	50%
Cycles per Burst	50
Duration	60 seconds
Bath Temperature	18°C
Extraction Solvent volume	50 µl
M220 Holder XTU	500414
M220 Holder XTU Insert	500489
microTUBE-130 Glass Beads Pre-Slit Screw-Cap (25)	520199

Note:

Recommended settings are subject to change without notice. Contact Covaris for application of this protocol to other Covaris Focused-ultrasonicator systems (such as S220, E220, and LE220).

See http://covarisinc.com/resources/protocols/ for updates to this document.

COVARIS SUPPLIES

Item	Materials	Description	Part Number
M220 Focused-ultrasonicator		M220 with computer and Software	500295

M220 Starter Kit

Item	Materials	Description	Part Number
truXTRAC MALDI-TOF MS Starter Kit			520194
	M220 Holder XTU	Holder	(500414)
	M220 Holder XTU Insert	Holder Insert for microTUBE-130 tubes	(500489)
	Prep Station	Prep Station for eight microTUBE-130 tubes	(500468)
	Centrifuge and Heat Block Adaptor	Centrifuge and Heat Block Adaptor for microTUBE-130 Screw-Cap	(500406)
	microTUBE Acoustical Cuvette	microTUBE-130 Glass Beads Pre-Slit Screw-Cap (25)	(520199)
	Eppendorf tubes 0.2 ml	Eppendorf #951010006	

Reorder items

Item	Description	Part Number
microTUBE Acoustical Cuvette	microTUBE-130 Glass Beads Pre-Slit Screw-Cap (25)	520199

ADDITIONAL MATERIALS (SUPPLIED BY USER):

- High purity water (e.g., HPLC/MS grade)
- Extraction Solvent (50% acetonitrile/35% formic acid/15% H_2O). Make up in advance using an amber glass container with screw-cap. Discard if not used after 30 days. For formic acid 98% mass spectrometry grade, use Fluka 94318-250 mL.
- Laboratory dry block heater (capable of heating to 100°C)
- Sterile, disposable 1 µl inoculating loops
- Centrifuge–fixed rotor (18,000 RCF)
- Variable pipette and tips (2.5 µl and 200 µl)
- Bruker MALDI Biotyper and mycobacteria library v1.0 or higher
- Bruker HCCA Matrix 8255344
- 0.2 ml Safe-Lock PCR tubes, 1000/pk Eppendorf #951010006

Values mentioned in this Quick Guide are nominal values. The tolerances are as follows:

- Temperature +/-2°C
- Sample volume +/- 5 µl

RISK AND SAFETY INFORMATION

The following protocol uses an organic solvent that according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) is considered a hazardous chemical. In the manufacturer's experience, the product has no harmful effect when used and handled according to instructions.

This sample preparation protocol for MALDI-TOF MS analysis may involve exposure to potentially dangerous biological material. Every person working with this protocol is responsible for following all of the necessary health and safety precautions to protect oneself and laboratory personnel.

All patient samples and cultures must be considered potentially infective. Only qualified laboratory personnel should perform this protocol. Personnel performing this protocol are responsible for taking and following all the necessary safety precautions for handling potentially pathogenic material. This would include the wearing of appropriate personal protective equipment such as a laboratory coat, safety glasses and gloves.

SAMPLE PREPARATION

Organisms should be grown on agar until colonies are visually present and experiencing freshly positive growth. Approximately 1 µL should be scraped from the plate using a disposable inoculation loop (4-8 mg).

OPERATING CONDITIONS

The Covaris AFA process focuses high intensity, high frequency acoustic energy through vessel walls and into a sample. The AFA energy is influenced by objects in the acoustic path from the surface of the transducer to the sample. For example, microscopic particles in the Covaris instrument water bath may scatter the acoustic energy from the sample and reduce processing efficiency.

WARNING: Replace water on a daily basis. In addition, if the daily use is high, replace after 100 samples during the day. When not in use, remove water from the M220.

For the M220 Focused-ultrasonicator, put the "Holder XTU" and the "Insert XTU microTUBE-130 µl" in place. Fill the water bath, use approximately 14 ml. Allow system to reach temperature.

Table 1 - "Mycobacterium MALDI-TOF MS" SonoLab parameters

	Power	Duty Factor	Cycles *per* burst	Time	Temperature
M220	40W PIP	50%	50	60 seconds	18°C

Prepare Heating Block

Dry block heaters should be preset at 100°C. Verify temperature with a calibrated glass thermometer.

PROTOCOL WITH HEAT INACTIVATION

1. Turn on the Covaris Focused-ultrasonicator, the associated computer, and launch SonoLab 7.2 software.
2. Load the "Mycobacterium MALDI-TOF MS" method in SonoLab if it is not already loaded, or create a new file (section 5.1, Covaris 010157 M220 Manual Rev E).
3. Ensure the water bath has the correct amount of water. Water should be to the top of the insert and just at the bottom of the hub of the microTUBE. The SonoLab control software will automatically enable the Run control when bath temperature is near the 18°C set point. The microTUBE is bar-coded. If you do not utilize the bar-code, manually label the plastic hub of the tubes for sample tracking using an indelible marker.
4. Place up to 8 tubes in the first two rows of the Prep Station (Figure 1).

NOTE: The Prep Station was designed for the Screw-cap microTUBE, it holds (8) eight tubes.

5. Add 100 µL of water to a 0.2 mL Safe-Lock PCR tube (Eppendorf 951010006); do not substitute this tube.
6. Add 1 µL inoculation loop of sample (4-8 mg) to the Eppendorf tube by shaking the loop until the cells disperse into the water.
7. Close the cap of each Eppendorf tube.
8. Transfer the Eppendorf tube to the laboratory block heater.
9. Heat the Eppendorf tube for 30 minutes at 100°C.
10. Carefully remove the Eppendorf tube from the block heater, allow to cool several minutes.
11. Centrifuge the Eppendorf tubes at 18,000 x g for five (5) minutes. A pellet should be visible.
12. Remove the cap from the Eppendorf tube.
13. Using a pipette, remove 95 µl of water above the pellet being careful not to disturb the pellet.
14. Add 50 µL of Extraction Solvent (50% acetonitrile/35% formic acid/15% H2O) directly onto the pellet in each Eppendorf tube.
15. Using a pipette, thoroughly re-suspend the cell pellet and transfer the 50 µL of Extraction Solvent and biomass to each microTUBE-130 with beads. By inserting the pipette tip through the split septum, you avoid removing the cap of the microTUBE.
16. Place a microTUBE with sample into the M220 instrument. Process each sample with AFA by running the "Mycobacterium MALDI-TOF MS" method. Run time is 60 seconds.
17. Remove each sample after processing, place the microTUBEs in the Prep Station.
18. Centrifuge each sample at 18,000 *x g* (batch) for 2 minutes using the Centrifuge and Heat Block Adaptors to support the microTUBE.
19. Remove the cap from the microTUBE. Using a pipette, draw several microliters of clarified sample, using the split septa of the microTUBE-130.
20. Spot 1 µl samples to the Bruker MALDI Biotyper plate and let dry. Add 1 µl of matrix (Bruker catalog # 8255344) onto the sample spot and let dry completely .

WARNING: All samples should be processed and spotted onto the MALDI-TOF target plate and analyzed within 60 minutes.

21. Follow the manufacturer's directions for completing the MALDI-TOF MS analysis.

Figure 1. Prep Station with microTUBEs

Table 2. Bruker MALDI Biotyper Scores* from *Mycobacterium smegmatis culture after a two-day growth on Middlebrook agar plates.*

#	Covaris truXTRAC	Bruker MycoEx
1	2.367	2.252
2	2.417	2.210
3	2.397	2.046
4	2.248	2.259
5	2.327	2.302
6	2.432	2.274
7	2.480	2.210
8	2.314	2.278
Average	**2.373**	**2.229**
cv%	3.13%	3.61%

*Using Bruker MALDI Biotyper mycobacteria database version 1.0

The above table represents typical scores obtained from culture plates from *Mycobacterium smegmatis* prepared with both the Covaris truXTRAC protocol and with the Bruker MycoEx protocol according to manufacturer's instructions.