PROTOCOL

Covaris

truXTRAC MALDI-TOF Mycobacteria Kit

MALDI-TOF Colony Sample Preparation Protocol for Mycobacteria

INTENDED USE

This protocol is for the preparation of Mycobacteria grown on agar plates or tube-slants. Colonies should be clearly visible (and not inoculum). Collect bacterial colonies using a sterile, disposable 1 µl inoculation loop (~4-8 mg of cells).

SUMMARY OF OPERATING CONDITIONS

AFA Instrument	**M220**
Peak Incident Power	40 W
Duty Factor	50%
Cycles per Burst	200
Duration	120 seconds
Bath Temperature	18°C
Volume	130 µL

Recommended settings are subject to change without notice.

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

SUPPLIES

Item	Covaris Materials	Description	Part Number
Focused-Ultrasonicator™		M220 with Computer and Software	500295
truXTRAC™ MALDI-TOF Mycobacteria Starter Kit			520165
	M220 Holder XTU	Holder for 520170	
	M220 Holder XTU Insert	Holder Insert for microTUBE™-130 µL	
	Prep Station	Prep Station for microTUBE Screw-Cap	
	Centrifuge Adaptor	Centrifuge Adaptor for microTUBE Screw-Cap	
	truXTRAC MALDI-TOF Mycobacteria Kit	microTUBE Acoustical Cuvette and truXTRAC Extraction Solvent	

Item	Covaris Materials	Description	Part Number
truXTRAC™ MALDI-TOF Mycobacteria Kit			520160
	microTUBE Acoustical Cuvette	microTUBE-130 Glass Beads No-Slit Screw-Cap (25)	
	truXTRAC Extraction Solvent	35% Formic Acid, 50% Acetonitrile, 15% H_2O (3 mL)	

ADDITIONAL MATERIALS (SUPPLIED BY USER):

- Sterile, disposable 1 µl inoculating loops
- Centrifuge–fixed rotor 18,000 RCF
- Variable pipette and tips –2.5 µL and 200 µL

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.

truXTRAC Extraction Solvent is classified as a hazardous chemical: SDS INFORMATION IS AVAILABLE AT http://covarisinc.com/resources/msds-sheets/

This sample preparation protocol for MALDI-TOF 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 an agar plate until colonies are visually present and experiencing healthy growth.

Approximately 1 µL should be scraped from the plate using a disposable inoculation loop (4-8 mg).

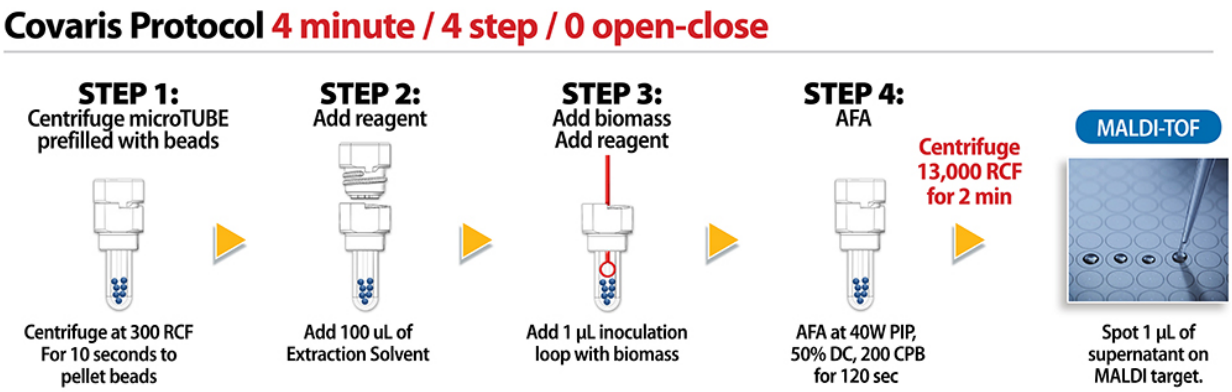
OPERATING CONDITIONS

The Covaris process focuses high frequency acoustic energy through vessel walls and into the sample and as such is influenced by objects in the acoustic path from the transducer surface to the fluid sample. For example, particles and bubbles in the water bath may scatter the acoustic energy from the sample. Please replace the water on a daily basis.

For M-Series Focused-ultrasonicators, fill the water bath with the Holder-XTU microTUBE in place until the water reaches the top of the holder. Allow the system to reach temperature.

OVERVIEW OF PROTOCOL STEPS

Colony



PROTOCOL

1. Once the computer and Sonolab 7.2 software is running, load the Mycobacterium protocol file if it is not already loaded. Insure the water bath has the right amount of water.
2. Centrifuge the pre-filled microTUBEs with beads at 3000 RCF for 10 seconds to pellet the beads. Use the centrifuge adapters to support the microTUBEs in the centrifuge.
3. The microTUBEs are bar-coded. If you do not utilize the bar-code, manually label the tubes for sample tracking using an indelible marker.
4. Place up to 4 microTUBEs in the front row of the Prep Station. The Prep Station has 8 places for microTUBES and has been designed to allow the user to keep track of un-processed and

processed samples. The station also enables one-handed opening of the microTUBE, and the back row of the Prep Station is configured to hold the microTUBE screw cap while adding sample.

5. Add 100 µL of truXTRAC Extraction Solvent (35% formic acid/50% acetonitrile/15% H_2O) to each microTUBE.
6. Add 1 µL inoculation loop of sample (4-8 mg) to the microTUBE by shaking the loop until the cells disperse into the Extraction Solvent.
7. Place a screw cap onto each microTUBE and tighten.
8. Place a microTUBE with sample into the M220 and AFA the sample at 40 PIP (W), 50% Duty Cycle (DC), 200 cycles per burst (cpb) for 120 seconds. Repeat the process until all samples have been processed. Once the sample has been processed, place the microTUBE in the second row of the Prep Station.
9. Centrifuge all samples at 18,000 *x g* for 2 minutes using the centrifuge adaptors to support the microTUBEs.
10. Spot 1 µL onto the target plate or slide. All of the samples should be processed and spotted onto the MALDI target within 60 minutes.
11. Follow the manufacturer's directions for completing the MALDI-TOF analysis.

TYPICAL OUTPUT READINGS: VARIATION

Table 3. Bruker Biotyper Scores from Mycobacterium smegmatis.

Sample	Bruker Score	bioMerieux Score
1	2.13	TBD
2	2.34	TBD
3	2.36	TBD
4	2.17	TBD
Avg	2.25	TBD
CV	0.05	TBD