MALDI Biotyper solutions preparation

"Organic Solvent": 50% aqueous acetonitrile (ACN), 2.5% trifluoroacetic acid (TFA)
Used for dissolving the standard (BTS) and matrix (HCCA)
Mix in a 100-mL glass bottle (work under the fume hood!):
19 mL water
1 mL TFA (1 ampule)
20 mL acetonitrile

Matrix solution in "Organic Solvent"
10 mg/mL HCCA in 50% ACN, 2.5% TFA
Good for 1 week at r.t.

Biotyper Standard solution (BTS) in "Organic Solvent"

1. Add 50 µL of 50% aq. ACN, 2.5% TFA
2. Dissolve by gently pipetting up and down 20 times; avoid generating bubbles
3. Let stand at room temperature for 5 minutes
4. Repeat pipetting up and down 20 times
5. Centrifuge for 2 minutes at maximum speed (13,000 to 15,000 rpm)
6. Aliquot 5µL into Eppendorf microfuge tubes (screw caps recommended) and store at -18 ^{o}C or below for up 5 months; do not refreeze once thawed; label with preparation date/initials

"70% Formic Acid" for the Formic Acid (FA) extraction protocol
Mix in a 20-mL glass vial (work under the fume hood!):
10 mL 98% formic acid
4 mL water

For 99% FA in 1-mL ampules, add 0.414 mL water for every 1 mL of 99% FA.

Other reagents for the FA extraction

- 100% 'ethanol' (EtOH)
- 100% acetonitrile (ACN)
- water

Direct transfer method (with optional FA)

1. Smear a thin film of biological material (single colony) directly onto a clean MALDI target using a pipette tip or a wooden toothpick. Use clean pipette tip or toothpick for each sample. Distribute the material evenly over the spot. A colony size of a pin head or poppy seed is generally enough.
2. Optional: Overlay each sample with 1 µL 70% FA. Adding FA improves the disruption of the cells, and is especially recommended for Gram-positive bacteria and yeasts.
3. Carefully overlay each sample with 1 µL of matrix. If using FA, allow FA to evaporate before adding the matrix solution to avoid spilling of the samples into the neighboring spots.
4. Allow the samples to dry at room temperature.

Store the agar plates at room temperature if further classification analyses are necessary. Storing cultures in a refrigerator will negatively affect the mass spectra quality.

Tube Extraction (FA extraction) Procedure

1. Add 300 µL of water to each microcentrifuge tube
2. Transfer a large, single colony of microorganism to the tube (more than one colony may need to be transferred if microorganism is small; chose isolated colonies); vortex thoroughly
3. Add 900 µL of EtOH; vortex thoroughly
4. Centrifuge at maximum speed (13,000 to 15,000 rpm) for 2 minutes
5. Decant EtOH; centrifuge for 2 minutes
6. Remove ALL excess EtOH with pipette (completely remove all ethanol; tubes may be left at room temperature to complete the evaporation process, if necessary)
7. Add 50 µL of 70% FA (if only a small amount of microorganism is available, use 10 µL FA)
8. Vortex thoroughly and let stand for approximately 5 minutes
9. Add 50 µL of 100% ACN (for small amount of microorganism use 10 µL); vortex thoroughly; NOTE: the volumes of 70% FA and ACN must be equal to achieve a final ACN concentration of 50%.
10. Centrifuge at maximum speed (13,000 to 15,000 rpm) for 2 minutes
11. Pipette 1 µL of supernatant onto steel target; avoid touching pellet at bottom of tube with pipette tip; air dry
12. Overlay with 1 µL of matrix and allow to dry at room temperature

Target cleaning solutions preparation

70% Ethanol
Mix in a squeeze bottle
30 mL water
70 mL ethanol

80% aqueous TFA
Mix in a microcentrifuge tube (work under the fume hood!)
1 mL TFA (1 ampule)
0.250 mL water

Target cleaning procedure

1. Transfer the MSP target to a crystallizing dish (8 x 4 cm) or a small stack of paper towels under the fume hood.
2. Overlay the surface of the target with 70% aqueous ethanol using squeeze bottle; let stand 5 minutes
3. Wipe surface with Kimwipe; rinse with water
4. Wipe surface of target with 70% aqueous ethanol and Kimwipe
5. Rinse the target with deionized (distilled) water and dry with Kimwipe
6. Cover the target with a layer of 100 µL of 80% aqueous TFA and wipe intensively (work under a chemical hood and wear chemical safe gloves!)
7. Rinse the target with deionized water and wipe it dry with a Kimwipe.
8. Let the target completely dry for at least 15 minutes at room temperature before use

Supplies and reagents

You can use any supplier as long as the reagents are of the specified grade. Catalog numbers were last checked on 08/13/2015

Target plate (before ordering, check if one is currently available for you to use)
MSP 96 target polished steel; Bruker Part No: 8280800, appr. cost $400

Bacterial Test Standard (BTS)
Bruker Part No: 8255343, 5 tubes per package; appr. 40 tests per tube

Formic acid (FA), 10 x 1-mL glass ampules, >99%
Fisher cat. no. PI-28905 Pierce Biotechnology Inc No.: PI28905

Trifluoroacetic acid (TFA), 10 x 1-mL glass ampules
Fisher cat. no. PI-28904 Pierce Biotechnology Inc No.: PI28904

Acetonitrile, LC-MS Grade, 1 L
Pierce (Thermo, Life Technologies); cat. number 51101

Water, LC/MS grade, 1 L
Fisher cat. no. PI-51140 Pierce Biotechnology Inc No.: PI51140

Matrix (alpha-cyano-4-hydroxycinnamic acid, HCCA), 1 g
Sigma cat. no. 70990-1G-F

Ethanol, Chromasolv LC grade, 1 L
no license required, contains 5% methanol and 5% isopropanol
Sigma cat. no. 270741-1L

Microcentrifuge tubes
Do not use ‘low-binding’ or silanized tubes; any plain polypropylene microcentrifuge tubes will work.