

## 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

### “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.

### Matrix solution

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

### Biotyper Standard solution (BTS)

1. Add 50  $\mu$ L 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 5 $\mu$ L into Eppendorf microfuge tubes (screw caps recommended) and store at -18  $^{\circ}$ C or below for up to 5 months; do not refreeze once thawed; label with preparation date/initials

### Other reagents for the FA extraction

- 100% ‘ethanol’ (EtOH)
- 100% acetonitrile (ACN)
- water

## Tube Extraction (FA extraction) Procedure

1. Add 300  $\mu$ L 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  $\mu$ L 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  $\mu\text{L}$  of 70% FA (if only a small amount of microorganism is available, use 10  $\mu\text{L}$  FA)
8. Vortex thoroughly and let stand for approximately 5 minutes
9. Add 50  $\mu\text{L}$  of 100% ACN (for small amount of microorganism use 10  $\mu\text{L}$ ); 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  $\mu\text{L}$  of supernatant onto steel target; avoid touching pellet at bottom of tube with pipette tip; air dry
12. Overlay with 1  $\mu\text{L}$  of matrix

## 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  $\mu\text{L}$  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 \$380

**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.