

## Sample Preparation Procedure for

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# Cereulide

## Acetonitrile Extraction

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### General Information

This protocol for the detection of the emetic toxin cereulide from colonies of *Bacillus cereus*, provided by CVUA Freiburg, is based on Döllinger et al. (2020) and Ulrich et al. (2019) [1,2], adapted and optimized to on-site conditions and introduced into routine practice using a Bruker Biotyper LT Microflex (Bruker, Bremen, Germany). The protocol is compiled as part of a master's thesis in the field of molecular biosystems in cooperation with Otto-von-Guericke University Magdeburg.

### Field of Application

Colony material from presumptive *Bacillus cereus*

### Chemicals and Material

- 2 ml reaction tubes
- Pipettes and tips for volumes from 1-500 µl
- 10 µl sterile inoculation loops
- Benchtop centrifuge
- Vortex-mixer
- Casein-Soy-Peptone agar (CASO)
- Acetonitrile (ACN)
- HCCA matrix solution

### References

- [1] Döllinger, Jörg; Schneider, Andy; Stark, Timo; Ehling-Schulz, Monika; Lasch, Peter: Evaluation of MALDI-ToF Mass Spectrometry for Rapid Detection of Cereulide from *Bacillus cereus* Cultures. Supporting Information.
- [2] Ulrich, Sebastian; Gottschalk, Christoph; Dietrich, Richard; Märklbauer, Erwin; Gareis, Manfred (2019): Identification of cereulide producing *Bacillus cereus* by MALDI-TOF MS. In: *Food-microbiology* 82, S. 75–81. <https://doi.org/10.1016/j.fm.2019.01.012>

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#### Extraction Procedure

- Cultivate presumptive *Bacillus cereus* on CASO at 25°C for 48 hours
- Solve at least 10 µl *Bacillus cereus* colony material in 100 µl ACN using a inoculation loop
- Vortexes subsequently
- Centrifuge at 4000 rcf in a benchtop centrifuge for 5 minutes
- Pipet 1 µl supernatant onto a target sample spot (we recommend spotting the supernatant in duplicate or triplicate)
- As soon as the sample spot has dried, overlay the sample with 1 µl HCCA matrix solution (to prevent oxidation reactions which might cause peak shifts)
- Allow the sample spot to air dry before analysis → MALDI measurement

#### MALDI-ToF MS Device settings

- Laser Power & Detector Gain: Calibrate your device in such way that it is optimized for measuring in this mass range (characteristic Cereulide peaks are: 1191 Da, 1175 Da [2])
- Evaluation mass range: 0 – 2000 Da
- Accumulation: MS / Parent Mode; sum up 700 satisfactory shots in 50 shot steps
- Movement: Random walk; shots at raster spot: 10
- Tipp: Cereulide dries on a small concentrated spot. In order to hit this spot, set your device to scan as much of the target area as possible.