MADLI-TOF/TOF Sample Preparation

Tryptic digest of gel lanes for analysis with MALDI-TOF:

- Be careful that skin scales or hair do not contaminate your sample. So wear hand gloves and tie up your hair.
- Reaction tubes have to be cleaned with 60 % (v/v) CH_3CN and 0.1 % (v/v) TFA. Afterwards the solution has to be removed completely followed by evaporation of the tubes under a fume hood. Alternatively, microtiter plates from Greiner® (REF 650161) can be used without washing. If you work with reaction tubes from Eppendorf, you do not need the wash step either.
- Cut out the protein lanes of a Coomassie-stained SDS-PAGE using a clean scalpel. Gel parts are transferred to the washed reaction tubes. If necessary, cut the parts to smaller slices
- Gel slices should be washed two times. Therefore add 200 µL 30 % (v/v) acetonitrile in 0.1 M ammonium hydrogen carbonate each time and shake lightly for 20 minutes till the gel slices are destained. Remove supernatant and discard to special waste
- Dry gel slices at least 30 minutes in a Speedvac.
- Rehydrate gel slices in 15 µL trypsin solution followed by short centrifugation.
    - Trypsin-solution: 1 µL trypsin + 14 µL 10 mM NH_4HCO_3
        - For this solution solubilize lyophilized trypsin in 200 µl of provided buffer and activate Trypsin for 15 minutes at 30 °C. For further use it can be stored at -20 °C.
- Gel slices have to be incubated 30 minutes at room temperature, followed by incubation at 37 °C overnight
- Dry gel slices at least 60 minutes in a Speedvac.
- According to the size of the gel slice, add 5 - 20 µL 50 % (v/v) ACN / 0.1 % (v/v) TFA
- Samples can be used for MALDI measurement or stored at -20 °C

Preparation and Spotting for analysis of peptides on Bruker AnchorChips:

- Spot 0.5 - 1 µL of sample aliquot
- Add 1 µL HCCA matrix solution to the spotted sample aliquots. Pipet up and down approximately five times to obtain a sufficient mixing. Be careful not to contact the AnchorChip. Note: Most of the sample solvent needs to be gone in order to achieve a sufficiently low water content. When the matrix solution is added to the previously spotted sample aliquot at a too high water content in the mixture, it will result in undesired crystallization of the matrix outside the anchor spot area.
- Dry the prepared spots at room temperature
- Spot external calibrants on the adjacent calibrant spot positions. Use the calibrant stock solution (Bruker's "Peptide Calibration Standard II", Part number #222570), add 125 µL of 0.1 % TFA (v/v) in 30 % ACN to the vial. Vortex and sonicate the vial.
- Mix the calibrant stock solution in a 1:200 ratio with HCCA matrix and deposit 1 µL of the mixture onto the calibrant spots
- Analyze samples in ultrafleXtreme by Bruker Daltonics.
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