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ClinProt MALDI Protocols

Guidelines for sample preparation
(How to protect your samples from contamination with keratin)

- **Clean your bench**
- **Try to avoid contact of samples and solutions with dust, skin or hair**
- **Wear gloves at all times**
- **All reagents should be prepared fresh**
- **Use ultra-pure water for all solutions**

HCCA Matrix (for peptides and small proteins on *AnchorChip*)

prepare a matrix solution with α-Cyano-4-hydroxycinnamic acid (HCCA) of 0.3 g / l in ethanol:acetone 2:1 (prepare fresh every day)

dilute **1 µl** of eluted sample in 10µl of matrix solution

spot **0.5-1 µl** of this solution to a *AnchorChip* target

DHAP (2,5 Dihydroxyacetophenone) Matrix (for proteins)

suspend 7.6 mg DHAP in 375 µl ethanol

add 125 µl (10 µmol) of DHAP solution (27 mg / 1.5 ml milliQ water)

vortex for 1 min at RT

sonicate for 15 min followed by vortexing for 1 min at RT

store the matrix in the dark for max. 1 week

DHAP for AnchorChip 600 µm

mix **2 µl** of magnetic bead eluate with 2 µl of 2% TFA

add **2 µl** of the DHAP matrix (above)

the matrix / analyte mixture should be mixed

spot **0.5-1 µl** of this solution to a 600 µm AnchorChip target, prevent air bubbles

HCCA Matrix (for *AnchorChip*)

to a **2 ml** eppendorf tube containing 2 mg of HCCA add 2 ml of 100 % acetone and mix for some minutes until complete solubilisation

pipette **4 ml** of ethanol in a Teflon tube and add the HCCA solution (2 ml) to this tube

this matrix solution has a concentration of 0.3 mg / ml in 2:1 ethanol / acetone

Cleaning of the *AnchorChip*

wash the target plate intensively under flowing hot tap water

wipe the target with acetone using a kimwipe

rinse with distilled water

wipe with methanol using a kimwipe, rinse with methanol

dry with air

ClinProt Calibrant Standards (CPS)

thaw an aliquot of CPS (approx 10 µl) at RT

mix 2 µl of CPS with 20ul of freshly prepared Matrix (HCCA)

pipette 0.5 µl of CPS/Matrix mixture on at least 8 calibrant positions on *AnchorChip*

let it dry

Calibration of MALDI TOF/TOF in the mass range 1-10 kDa

after accumulation of approximately 150 shots calibrate in the flexControl Method with the ClinProt Standard

use the sum buffer for calibration

try to use all peptides from the standard with the quadratic equation

save the calibration to the flexControl method