

2D Electrophoresis

Sample desalt/cleanup - ZipTip (PSC 537)

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The TFA has a limited shelf life. There is different information about how long to use TFA once opened. Replace the TFA if it turns yellow or after 4 - 6 months. In the Supplies section is order information about a 0.5 ml ampule of TFA, so you don't have to toss too much.

One of the biggest problems with protein yield after in-gel digestion and ZipTip is when the peptides stick to the plastic - tips, tubes or plates. Included in the Buffers and Supplies section are some low-bind products that might help. Also, be careful not to dry the peptides completely. This can't be avoided with a microplate, though. So, again, think about using a low-bind plate.

De-salting (uC18) for TOF/TOF (Pierce lab)

1. Add 1.5ul neat formic acid and vortex.
2. Add 8.5ul 0.1% TFA, vortex at low speed for 1 minute, and spin down. Set aside until tip is prepped.
3. At max volume setting (10ul) aspirate and discard wetting solution twice.
4. Equilibrate by aspirating and discarding 0.1% TFA 10X.
5. Bind by aspirating and dispensing sample 7X.
6. Wash bound sample 10X with 0.1%TFA. Aspirate and discard each wash.
7. Add 0.7ul elution solution into the sample microfuge tube cap. Aspirate and elute the entire droplet of elution solution that is in the cap of the tube. Repeat 5X (no air). On the final aspiration, draw up the entire volume and maintain pressure (do not take up any air or the sample will be lost). Dispense the sample until a droplet forms at the pipette tip and touch the droplet (not the pipette tip) to the TOF/TOF plate.
8. Allow to dry and spot .3ul alpha matrix onto each spot. Create a droplet on the end of the pipette tip and touch the droplet to the spot.

De-salting/Cleanup ZipTip (CDC)

1. Add 20ul 0.1% TFA to sample to dilute the SDS which can be a problem.
2. At max volume setting (10ul) aspirate and discard wetting solution 3X.
3. Equilibrate by aspirating and discarding 0.1% TFA 2-3X.
4. Bind by aspirating and dispensing sample 20X.
5. Wash sample 10-20X with 0.1%TFA to flush salts from peptides which are on ZipTip resin.
6. Aspirate and dispense 3X with CHCA:ACN mixture. On the last dispense, spot on the MALDI plate.

Buffers and Solutions

Wetting solution

50% ACN in milli-Q water– 1ml

500ul ddH₂O

500ul ACN

0.1% TFA in milli-Q water – 1ml

1ml ddH₂O

1ul TFA

Elution solution TOF/TOF

(0.1% TFA / 70 % ACN in milli-Q water) – 1ml

700ul ACN

300ul ddH₂O

1ul TFA

CHCA:ACN mixture

1.5 ul CHCA

0.5 ul ACN

Supplies

1. Trypsin

Quantity/Unit: 100 ug/vial (5 X 20ug)

Vendor: Promega

Catalog#: V511A

2. TFA (Trifluoroacetic acid

Quantity/Unit: 1 Pkg (5 X 0.5 ml)

Vendor: Aldrich

Catalog#: 308897-1PAK

3. Siliconized microfuge tubes, 0.5 ml (\$50.35/PK)

Quantity/Unit: 500/PK

Vendor: Applied Biosystems

Catalog#: AM12350

4. Siliconized microfuge tubes, 1.5 ml (\$40/PK)

Quantity/Unit: 250/PK

Vendor: Applied Biosystems

Catalog#: AM12450

5. Low Retention microfuge tubes, 0.6 ml (\$25/PK)

Quantity/Unit: 1000 (10 bags of 100)

Vendor: CLP, Continental Lab Products

Catalog#: 3435.S3

7. Low Retention microfuge tubes, 1.5 ml (\$14/PK)

Quantity/Unit: 500

Vendor: CLP, Continental Lab Products

Catalog#: 3445.S3

8. Low Retention pipet tips, Racked and Pre-sterilized, 200 ul(\$25.50/PK)

Quantity/Unit: 10 trays of 96

Vendor: CLP, Continental Lab Products

Catalog#: 2102.YS

9. Low Retention pipet tips, Racked and Pre-sterilized, 10 ul(\$25.50/PK)

Quantity/Unit: 10 trays of 96

Vendor: CLP, Continental Lab Products

Catalog#: 2142.S

10. Low Bind microfuge plates, conical, Pre-sterilized

Quantity/Unit: 100

Vendor: PGC

Catalog#: 81-6664-56