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STAGE (STop And Go Extraction) C18 tips for Desalting and Clean Up

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Protocol status: Working
We use this protocol and it's working

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Troubleshooting

Preparing Solutions

1 Abbreviations:
- ACN: acetonitrile
- TFA: trifluoroacetic acid
- FA: Formic Acid
- MeOH: Methanol

2 1% TFA

3 2-propanol

4 Buffer A: 2% ACN with 0.1% TFA
To make 100 mL
- 10 mL 1% TFA
- 2 mL ACN
- 88 mL Milli-Q water

5 Buffer B: Anywhere from 30-80% ACN with 0.1% TFA

General peptides are eluted with 60% ACN with 0.1% TFA (***this is also used for tip conditioning***)
To make 100 mL
- 10 mL 1% TFA
- 60 mL ACN
- 30 mL Milli-Q water

Immunopeptides are eluted with 30% with 0.1% TFA
To make 100 mL
- 10 mL 1% TFA
- 30 mL ACN
- 60 mL Milli-Q water

6 Mass Spectrometry Buffer A: 2% ACN with 0.1% FA

Note

It is important to use FORMIC ACID for mass spectrometry solvents

Making tips

7 Prepare as many tips as necessary by one of two methods: C18 empore filter or frit + C18 powder

8 The filter/frit will act to keep the powder in the P200 pipette tip. The frit has a pore size, but this is much larger than the pore size of C18 filter.

9 Punch out a disk using a 17G flat-tipped syringe. Use C18 Empore filters or a SPE frit.
- Careful not to squish the frit as this will cause a blockage
- The easiest way to do this is to line the syringe up gently on top of the frit, then hammer the syringe down (rather than twisting and manually pushing).

10 Eject the frit/frit disk into a P200 pipette tip with an unfolded paper clip

11 Ensure that the filter/frit is secrely wedged in the bottom of the tip.
- The frits are much harder than the C18 filter, therefore, more resistant to folding at the bottom of the tip.

Adding Powder

12 Using a multi-channel pipette resevoir, add in C18 powder and wet it with methanol to create a slurry

Note

This step is optional if a C18 membrane was used.

13 Using a pipette, add C18 slurry on top of the filter/frit

14 Compact the slurry and remove the ethanol by forcing the methanol from the slurry. This can be done with a Luer lock syringe fitted to the tip of the pipette tip. Alternatively, this can be done by centrifuging the tips at 800g. However, when conditioning the tips it is

advised to manually force the solvent through to check the tightness/loseness of the frit + slurry.

15 A different amount of C18 powder will be added each time as the slurry is not homogenous. Add slurry up to a desired height. 10mg of C18 powder is ~11mm above the frit.

Sample Preparation

16 If your sample is dry, reconstitue it in Buffer A. If the sample is aqueous, acidify it with 1% TFA to a pH <2.5. Reconsitute in 20 µL Buffer A or up to 100 µL Buffer A depending on the sample concentration.

> Note
>
> It is important to acheive a pH less than 2.5 to ensure all amino acids are in their protonated form

17 If the concentration of the sample is unknown, determine it by NanoDrop

> Note
>
> 10mg of C18 powder can handle ~ 200-500µg of protein from a full cell lysate

Tip Wetting

18 Wet the tips with 50 µL 2-propanol

Tip Conditioning

19 Condition the tip with 100 µL Buffer B (50-60% ACN with 0.1% TFA)

Tip Hydration

20 Hydrate the tip with 100 µL Buffer A

Sample Loading

21 Pass the reconstituted sample slowly through the tip

Note

Optional: collect flow through and store at -20˚C in case of improper tip usage

Tip Washing

22 Wash the tip with 200 µL 1% TFA

Note

Optional: collect flow through and store at -20˚C in case of improper tip usage

Note

Optional: If there is not time to elute and dry the sample, the peptides can be stored "dry" on the tip at 4˚C overnight or -20˚C for longer periods. This is a better option than eluting the peptides and storing in
Buffer B.

Sample Elution

23 Elute the sample with 2X 100 µL Buffer B (your selected % ACN) into a 0.5 mL microtube or a 96-well plate

Drying

24 Dry the sample completely in a SpeedVac and recostitute in the Mass Spec Buffer