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ZipTip sample clean-up

- Use µ-C18 ZipTip for cleaning up peptide samples
- Your sample must be in an aqueous buffer to bind to the ZipTip. If your sample is dry, rehydrate it in 1% Formic Acid/ 2% acetonitrile.
- Use only USA scientific 1.5 ml Seal-Rite microcentrifuge tubes, 500/bag (polypropylene), catalog # 1615-5500. If you use other tubes, plasticizers may leach into the samples and interfere with mass spec analysis.
- Prepare solutions fresh daily.
- Use a new ZipTip for every sample.
- Prepare 4 µl aliquots of elution buffer (60% acetonitrile/0.1% formic acid) for each sample before beginning (to avoid contamination).
- Avoid drawing air through the tip during the procedure (from equilibration to elution). If you find that you make bubbles in the tip, try pulling the buffers in more slowly.

Set pipetteman to 10 µl and attach the ZipTip:

- **Equilibrate the ZipTip:**
  Using the following buffers, pipette up the buffer and then discard it in the waste
  - 3 X with 100% acetonitrile
  - 3 X with 0.1% formic acid.

  These steps act as a gradient for the mini-column, which wets the resin and conditions it to be ready to bind peptides.

- **Load the peptides:**
  Load the sample by pipetting the protein digest up and down (discarding it back into its tube)
  - 10 X with digest

- **Wash the ZipTip:**
  Using the following buffer, pipette up the buffer and then discard it in the waste
  - 6 X with 0.1% formic acid

- **Elute the sample:**
  Elute the peptides by pipetting the ZipTip up and down in the elution buffer (back into its tube)
  - 10 X in the 4 µl 60% acetonitrile/0.1% formic acid (already aliquoted) The organic phase elutes the peptides off the resin and into the buffer. Now the sample is desalted and is concentrated in 4 microliters of solution appropriate for analysis by MALDI-TOF and ESI-Q-TOF.

If you are not going to do mass spec today, freeze your samples at –80°C until needed.