**ZIP TIP® PROTOCOL (adapted from Millipore procedure)**
See http://www.millipore.com/catalogue/module/c5737

Use C18 resin for peptide desalting
Use C4 resin for protein desalting

- *Prepare fresh solutions fresh weekly*; write the date, solution # and solvent composition on all tubes

- Prepare a 10% aqueous solution of trifluoroacetic acid: to 90 uL of water add 10 uL of neat, 99% purity (or higher) trifluoroacetic acid using a dedicated Hamilton gas-tight glass syringe. Rinse the syringe with 3x with nanopure water followed by 3x rinse with methanol before storing the syringe.

**Prepare the following solutions in 1 mL Eppendorf tubes:**

| Solution # | Composition | Volume (µl) Acetonitrile | Volume (µl) nanopure $H_2O$ | Volume (µl) 10% $TFA_{aq}$ | Total Volume (µl) |
|---|---|---|---|---|---|
| **1** (hydration solution) | 80:20, ACN:$H_2O$, 0.1% TFA | 800 | 190 | 10 | 1000 |
| **2** (wash solution) | 0.1% TFA in $H_2O$ | 0 | 990 | 10 | 1000 |
| **3a** (peptide elution solution) | 40:60, ACN:$H_2O$, 0.1% TFA | 400 | 590 | 10 | 1000 |
| **3b** (protein elution solution) | 75:25, ACN:$H_2O$, 0.1% TFA | 750 | 240 | 10 | 1000 |
| **R** (reconstitution solution for dried samples) | 5:95, ACN:$H_2O$, 0.1% TFA | 50 | 940 | 10 | 1000 |

Prepare three 0.6 ml Eppendorf tubes (for desalting of a single sample) as follows:

| Tube label: | Contents | Application |
|---|---|---|
| waste | (empty) | |
| wash (or $H_2O$) | ~60 µl of solution **2** | |
| [sample name] | 1.3 µl of solution **3a** or **3b** (place tube ice; keep lid open) | C18 or C4 Zip Tips® |

**Sample preparation**

1) Reconstitute dried samples with 13 µl of **Reconstitution solution;** vortex for 45 seconds and centrifuge at 4000 x g for 2 minutes
2) **CHECK SAMPLE pH:** if pH is **>3**, add a very small amount (for example: 0.3 µL) of 10% TFA; vortex; centrifuge; re-check pH, adjust pH to **≤3**

**ZipTip® Procedure**

1) Set a P10 pipettor to 10 µl, place ZipTip on P10 pipettor
   Important:
   - Pipet *slowly* to avoid introducing air into the packing material and to maximize binding; this is very important
   - Do not aspirate air through the ZipTip® once the procedure has begun
2) **HYDRATE**:
   a. Aspirate 10 µl of solution **1**, discard to waste; repeat
   b. Aspirate 10 µl of solution **2** discard to waste; repeat
3) **LOAD**: Slowly aspirate 10 µl of sample and expel the liquid back into the tube; repeat 5-6 times
4) **WASH**: Aspirate 10 µl of solution from **wash/$H_2O$ tube** and expel into **waste tube**; repeat wash step 4-5 times
5) **ELUTE**: Aspirate and expel the 1.3 µl aliquot of elution solution (**3a** or **3b**) that was placed on ice; repeat **2 times (**expel solution into the sample tube each time)
   - Careful! pipettor setting is 10 µl but aspiration volume is 1.3 µl; do not fully release pipettor plunger during the elution steps
6) **FOR MALDI-TOF Analysis:** Add 0.7 µl matrix solution (CCA (α-cyano-4-hydroxycinnamic acid) for peptides or sinapinic acid for proteins); mix; spot solution on MALDI target.
   *Alternative elution procedure: replace elution solution with matrix solution*