

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 µL of water add 10 µL 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 ₂ O	Volume (µl) 10% TFA	Total Volume (µl)
1 (hydration solution)	50:50, ACN:H ₂ O, 0.1% TFA	500	490	10	1000
2 (wash solution)	0.1% TFA in H ₂ O	0	990	10	1000
3a (peptide elution solution)	60:40, ACN:H ₂ O, 0.1% TFA	600	390	10	1000
3b (protein elution solution)	75:25, ACN:H ₂ O, 0.1% TFA	750	240	10	1000
R (reconstitution solution for dried samples)	5:95, ACN:H ₂ O, 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 ₂ O)	~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 and centrifuge
- 2) **CHECK SAMPLE pH**: if pH is >3, add a very small amount (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/H₂O 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) 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