

Stage Tips C18 for sample desalting:

Steps:

- 1) Conditioning the Stage Tip with 100 μ L of 100% methanol and waste it.
- 2) Equilibrate the Stage Tip with 100 μ L 0.1% formic acid and waste it.
- 3) Load the sample 2x times (you can collect the sample).
- 4) Wash the Stage Tip with 100 μ L of 0.1% formic acid (waste) – Repeat this step at least 10 times.
- 5) Elute with 100 μ L of 80% acetonitrile containing 0.1% formic acid.
- 6) Dry the samples.

Important information:

- 1) See the video to learn how to pack de membranes.
- 2) Two membranes of C18 (EMPORE™ - 3M) can be packed to load 10 μ g of digested proteins.
- 3) The samples must be at $\text{pH} \leq 3$. Formic acid can be used at final concentration of 0.1%. The samples can have a maximum of 150 μ L.
- 4) For each step in the centrifuge, use 2,000 rpm (Eppendorf 5417R) during 2 min.
- 5) The membrane can not dry.
- 6) If after centrifugation still remains a small volume in the tip, you can repeat the centrifugation by increasing the time and optimize according to your conditions (it can be applied for all steps).

Reference:

Ishihama et. al., Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using Stage Tips, Nature Protocols, vol.2, n.8, p.1896-1906, 2007.