

## Desalting with SepPak 96-well plate

This protocol is intended for estimated peptide amounts between 10 µg to 300 µg. If peptide amount is lower, use Pierce C18 tips (up to 8 µg or 80 µg, Thermo Fisher Scientific 87782 or 87784) according to manufacturer's instructions.

### Notes:

Sample should be acidic (0.5–1 % TFA, pH ~2).

Use vacuum pump and Waters manifold to dry wells. Do not use more than -0.1 bar vacuum.

Wells might dry unevenly. Wells that have already dried can be temporarily covered to speed up the drying of the remaining wells.

This same protocol can be used with Waters Sep-Pak C18 1 cc Vac cartridges (WAT054955).

### 1. MATERIALS

Methanol, HPLC-grade

Acetonitrile. LC-MS grade

Formic acid (FA), Supelco 1.00264.100

Trifluoroacetic acid (TFA), VWR 153112E

Sep-Pak tC18 96-well plate, Waters 186002321

96-well sample collection plate, Waters WAT058958

MilliQ-water

### 2. SOLUTIONS

Equilibration solution 1: 100% methanol

Equilibration solution 2: 80% acetonitrile in 0.1% TFA

Wash solution: 0.1% TFA

Elution solution: 50% LC-MS grade acetonitrile in 0.1% FA

### 3. DESALTING

1. Wet Sep-Pak 100 mg tC18 96-well plate (Waters part number: 186002321) with **1 ml of equilibration solution 1** (100% methanol)
2. Equilibrate with **1 ml of equilibration solution 2** (80% ACN in 0.1% TFA).
3. Equilibrate with **2 x 1 ml of wash solution** (0.1% TFA).
4. Apply the sample. Collect the flow-through and reapply it – use a clean collection plate.

5. Wash with **3 x 1 ml of wash solution** (0.1% TFA).
6. Elute the peptides with **1 ml of elution solution** (50 % ACN in 0.1% FA) – use a clean collection plate.

#### **4. SAMPLE PREPARATION FOR MS**

1. If concentration measurement is needed, Qubit can be used to measure concentration directly from eluate.
2. For other concentration measurements, dry desalted samples in SpeedVac and reconstitute in 0.1% FA.