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**Protocol status:** Working
We use this protocol and it's working

# Proteolytic Peptides from Conditioned Media Desalting with C18 Hydrophilic–Lipophilic Balance (HLB) Cartridges

Forked from Proteolytic Peptide Desalting with C18 Hydrophilic–Lipophilic Balance (HLB) Cartridges

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ABSTRACT

Desalting of proteolytic peptides from conditioned media elution using $C_{18}$ Hydrophilic–Lipophilic Balance (HLB) Cartridges in preparation for downstream proteomic profiling.

For desalting of proteolytic peptides from conditioned media Step 4 is modified as we typically reconstitute in an appropriate volume of 0.2% FA rather than by weight/volume due to the input material for digestion also being based on a proportion of the volume of concentrated conditioned media.

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MATERIALS

- Oasis HLB 1cc cartridges, 10 mg sorbent (Waters, Cat. #WAT058882)
- Vacuum manifold
- Centrifuge
- Centrifugal vacuum concentrator
- 1.5-mL microcentrifuge tubes
- Condition buffer/Elution buffer: 50% acetonitrile (ACN), 0.2% formic acid (FA) in water
- Equilibration buffer/Wash buffer: 0.2% formic acid (FA) in water
- HPLC-grade water

1 Centrifuge the samples at 1,850 x *g* for 5 minutes at room temperature to pellet insoluble material.

2 Desalt the samples using Oasis HLB solid-phase extraction cartridges placed on top of a vacuum manifold as follows:

2.1 Condition each cartridge two times with 800 µL of the condition buffer (50% ACN, 0.2% FA).

2.2 Equilibrate each cartridge three times with 800 µL of the equilibration buffer (0.2% FA).

2.3 Load the peptide samples.

2.4 Wash each cartridge three times with 800 µL of the wash buffer (0.2% FA).

2.5 Elute peptides with 800 µL of the elution buffer (50% ACN, 0.2% FA) followed by a further 400 µL of the same elution buffer.

3 Vacuum dry the eluted peptide solution in a centrifugal vacuum concentrator.

4 Reconstitute the dried peptides in an appropriate volume of 0.2% FA and thoroughly mix the solution.

5 Centrifuge at 12,000 x *g* at room temperature for 2 min, and store at -20°C.