thermo scientific

APPLICATION BRIEF 21959

SOLA high pH reversed-phase peptide fractionation by offline SPE

Authors

CCS Center of Excellence (CoE)
Application Scientists
Thermo Fisher Scientific

Keywords

Peptide Fractionation, Proteomics, Solid Phase Extraction (SPE)

Materials required

- Trifluoroacetic acid (TFA)
- Acetonitrile (ACN), LC-MS Grade
- Water, LC-MS Grade
- Triethylamine
- SOLA HRP SPE Cartridges, 10 mg
- Positive pressure or vacuum manifold for SPE cartridges
- Low protein binding microcentrifuge tubes

Introduction

High-pH reversed-phase chromatography is a robust method of peptide fractionation that separates peptides by hydrophobicity and provides excellent orthogonality to low-pH reversed-phase LC-MS gradients. In contrast to strong cation exchange (SCX) fractionation, high-pH reversed-phase fractions do not require an additional desalting step before liquid chromatography-mass spectrometry (LC-MS) analysis.

This principal can be applied to create a second dimension of separation when dealing with complex mixtures such as bioanalytical analysis of peptides. Cell lysates are extremely complex and subfractionating peptides can increase analytical depth and increase the detection of lower abundance components. This subfractionation can be done simply offline using solid phase extraction.

The range of Thermo Scientific™ SOLA™ Solid Phase Extraction (SPE) products use innovative frit-less SPE technology to eliminate the issues associated with traditional loose-packed SPE formats. SOLA HRP's polymeric sorbent bed allows for the use of high-pH solvents that would damage traditional silica-based SPE cartridges. Combining the support material and active media components into a solid uniform sorbent bed provides stable and controllable flow through characteristics and has an added advantage when dealing with viscous biological samples, as it prevents blocking and is also amenable to throughout processing due to 96 well plate formats.

ThermoFisher SCIENTIFIC

Important notes

- This protocol describes a fractionation using SOLA HRP SPE cartridges or SOLA HRP SPE 96 well plates with a 10 mg bed weight. Sample and solvent volumes may be adjusted for use with Thermo Scientific™ SOLAμ™ HRP 96 well plates with a 2 mg bed weight.
- Use low protein-binding microcentrifuge tubes to ensure maximum sample recovery.

Protocol

1. Condition SPE cartridge with 500 µL acetonitrile.
2. Equilibrate with two 500 µL aliquots of 0.1% TFA in water.
3. Load sample equivalent to approximately 10 µg protein digest in 600 µL 0.1% TFA in water.
4. Wash with 500 µL water.
5. Elute 8 total fractions of 2 × 250 µL (500 µL total for each fraction) using the elution solvents in the following table – Collect each 500 µL fraction.
6. Evaporate the liquid contents of each fraction and re-constitute in 2% ACN/0.05% TFA in water prior to analysis.

Fraction number	Acetonitrile (%) in 0.1% Triethylamine
1	5.0
2	7.5
3	10.0
4	12.5
5	15.0
6	17.5
7	20.0
8	50.0

Related Thermo Scientific products

60109-001 **SOLA HRP SPE cartridges,** 10 mg/1 mL, 100 pack
60309-001 **SOLA HRP SPE 96 well plate,** 10 mg/2 mL

Current versions of product instructions are available at separatedbyexperience.com/chromexpert.

Learn more about SOLA and SOLAμ Solid Phase Extraction at **thermofisher.com/sola-spe**

ThermoFisher SCIENTIFIC

AB21959-EN 0819S