Procedure – Disposable SCX Cartridge SPE

Application: Detergent removal and/or simple SCX fractionation of peptides

Materials: SPE SULFOETHYL Aspartamide Cartridge (Nest p/n PSPESE1203)
- Milli-Q Water
- Formic Acid – 88% or higher for adjusting pH
- SCX Diluent – 25%ACN/Water pH=3.0
- SCX “A” Buffer – 10mM KH₂PO₄/25%ACN pH = 3.0
- SCX “B” Buffer - 10mM KH₂PO₄/1M KCl/25%ACN pH = 3.0

Prepare: Equilibration/Loading/Rinse Buffer = 1:1 dilution of SCX Diluent and SCX “A” Buffer (5mM KH₂PO₄/25%ACN pH = 3.0)

Elution Buffer = 1:1 dilution of SCX “A” Buffer and SCX “B” Buffer (10mM KH₂PO₄/500mM KCl/25%ACN pH = 3.0)

Procedure: -- Reconstitute sample in 1ml Equilibration Buffer. Adjust pH to 2.5-3.0 with formic acid if necessary.
-- Wet cartridge with 2ml Milli-Q water.
-- Pass 1ml Elution Buffer through cartridge at 1-2 drops/second.
-- Wash cartridge with 2ml Milli-Q water.
-- Note: From here on, minimize the amount of air entering the cartridge as possible! Equilibrate the cartridge by passing 5ml Equilibration Buffer through at 1-2 drops/second.
-- Load sample onto the cartridge slowly at no faster than
1 drop/second. Collect the effluent.

-- Rinse the cartridge with 3ml Equilibration Buffer and collect
the effluent in the same tube as above. Allow the buffer to
flow though to the top of the cartridge bed.

-- Elute the sample by passing 1.5 ml Elution Buffer through the
cartridge at ~ 1drop/second. Collect the effluent in a clean
2ml centrifuge tube. Dry in Speed-Vap.

**Notes:** Simple SCX fractionations can be done by making various dilutions of
SCX “A” Buffer and SCX “B” Buffers and eluting the cartridge in 1.5ml
increments of increasing KCl concentration, e.g. 25mM, 75mM,
150mM, 250mM and 500mM. Collect each fraction in a separate 2ml
centrifuge tube.

Samples with large amounts of salt may require desalting by C18 SPE
or other means prior to performing the above procedure.

Checking/adjusting the pH prior to SCX is important to achieving the best
recovery.

Do not reuse the cartridge!