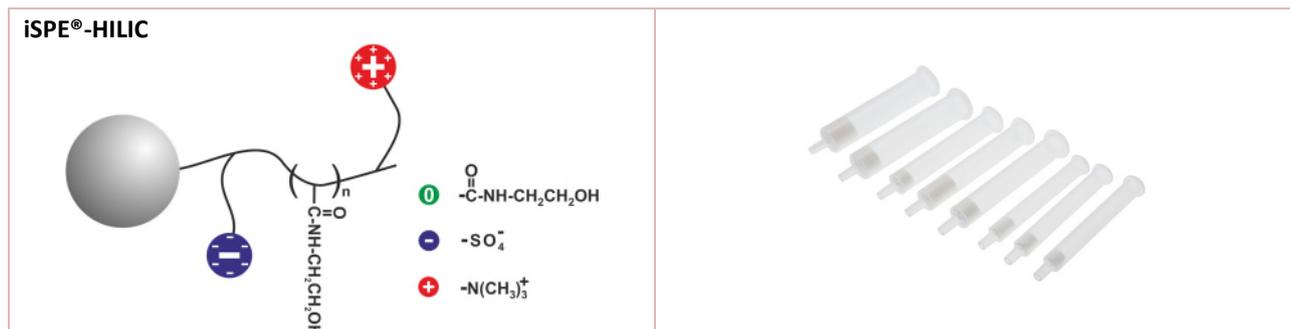


## Product Description

iSPE®-HILIC cartridges are designed for the purification of polar and hydrophilic compounds using Hydrophilic Interaction Liquid Chromatography (HILIC). The packed stationary phase is charge modulated hydroxyethyl amide silica particles which are covalently bonded with neutral, positively charged, and negatively charged hydrophilic functional groups. Therefore, the separation mechanism with iSPE cartridges is based on a combination of hydrophilic interaction, hydrogen bonding, and weak electrostatic interactions. They have unique separation selectivity and high retention capacity. Figure 1 presents the surface chemistry of iSPE®-HILIC and its available formats.



## Cartridges

The iSPE-HILIC is made from polypropylene and has 20 µm porous polypropylene frits to retain the material. The bonded silica particles are in 50 µm in particle size and 60 Å pore size. The surface area is about 250 m<sup>2</sup>/g.

Disposal of iSPE cartridges should follow the local authorities and regulations.

## Suggested Protocol for using iSPE-HILIC (1 mL, 50 mg cartridge, P/N 200.001.050)

### 1. Condition/Equilibration:

- Wash the SPE with 0.2-0.5 mL water or a suitable buffer (e.g., ammonium acetate/format).
- Wash the SPE with 0.5-1 mL organic solvent (e.g., acetonitrile).

### 2. Sample load:

- Preferably dissolve the sample in a solution containing 50-95% (v/v) of acetonitrile or other organic solvent
- Apply the sample (<0.2 mL) into the SPE cartridge and let it stay for 1 minute.
- Remove the solution by vacuum or push by an external pressure at a rate about 1-2 drops/second.

### 3. Wash:

- Apply the sorbent with 0.5-1 mL acetonitrile or suitable acetonitrile/buffer solution.
- Remove the wash solution by vacuum or push by an external pressure.

### 4. Elution:

Elute the polar compounds with a small volume of water or a solution containing acetonitrile or other organic solvent.

### 5. Preparation of final sample:

- Reconstitute the final SPE eluate prior to HPLC separation. If HILIC is used, it's important to make the injection sample have as much organic solvent as possible in order to obtain higher efficiency due to the peak compression principle.

## Solvents

For HILIC separations, water and buffer solutions are strong eluents and organic solvents are weaker. Acetonitrile is the most favorable solvent. The relative solvent strength for HILIC is: THF < Acetone < Acetonitrile < Isopropanol < Ethanol < Methanol < Water. Contrary to reversed phase chromatography, polar compounds have increased retention when increasing the proportion of organic solvent in mobile phase.

## Buffers and Additives

Solutions of 2-50 mM ammonium format and ammonium acetate are the best buffers for HILIC with MS detection. Their pH can be adjusted by adding formic acid, acetic acid, or ammonia. Phosphate buffers should be used with extra caution at lower concentrations due to the lesser solubility of sodium, potassium, and phosphate in HILIC mobile phases, which contain high concentrations of organic solvent. TFA and ion-pair reagents will change the selectivity of HILIC separations and often interfere with MS detection. Thus they should be avoided or used consciously to reduce the polarity of amines and the protonation of carboxyl groups for a specific purpose (i.e. isolation of glycopeptides from peptide digests).

### Samples

It's highly recommended to do sample preparations with mobile phase or solutions with similar ionic strength and concentration of organic solvent. More organic solvent in the sample solution will binding capacity and improve the recovery. Complex samples such as plasma or urine should be treated with a high proportion of organic solvent to precipitate proteins and salts, and filtered with 0.45 or 0.22 µm syringe filters that are compatible with organic solvents.

### Trademarks

iSPE® is a registered trademark of HILICON AB. All rights reserved 2014-2016.

### Order Information

P/N	Product Description	Unites/Box
200.001.0025	iSPE-HILIC SPE Cartridge, 1 mL, 25 mg, 50 µm particle	100
200.001.0050	iSPE-HILIC SPE Cartridge, 1 mL, 50 mg, 50 µm particle	100
200.001.0100	iSPE-HILIC SPE Cartridge, 1 mL, 100 mg, 50 µm particle	100
200.003.0100	iSPE-HILIC SPE Cartridge, 3 mL, 100 mg, 50 µm particle	50
200.003.0200	iSPE-HILIC SPE Cartridge, 3 mL, 200 mg, 50 µm particle	50
200.003.0500	iSPE-HILIC SPE Cartridge, 3 mL, 500 mg, 50 µm particle	50
200.006.0500	iSPE-HILIC SPE Cartridge, 6 mL, 500 mg, 50 µm particle	25
200.006.1000	iSPE-HILIC SPE Cartridge, 6 mL, 1 g, 50 µm particle	25