



PolyLC INC.

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Instructions for Item# SPEHY1201-SYR66: Hydrophilic Interaction Chromatography (HILIC)

APPLICATIONS: Universal trapping and concentration of polar small solutes; removal of salts and detergents. Elution order is least to most polar (the opposite of reversed phase). The capacity of this item is for samples up to 5 ml (larger samples can be loaded in portions provided the solvent promotes good binding). For samples < 1 ml, use our TopTips™.

RECOMMENDED SOLVENTS:

Binding Solution: 10 mM ammonium acetate or formate, pH 3.5, with 85-90% ACN. Unbuffered 0.1% formic acid can be used in place of the ammonium formate, but retention will not be as reliable.

Releasing Solution: Same but with 0-10% ACN.

CONDITIONING PROCEDURE:

- 1) Condition the cartridge with 5 ml of Releasing Solution (added in two portions), using a vacuum manifold to promote flow.
- 2) Equilibrate the cartridge with two 5-ml portions of Binding Solution.

SAMPLE BINDING:

IMPORTANT: To insure good binding, sample should contain about the same level of organic solvent as the binding solution.

Add sample slowly in order to give polar solutes time to be adsorbed to the stationary phase. It is recommended that it be added in portions of 1.2 ml, left for 1-2 minutes, then vacuum applied sufficient to pull the next portion of 1.2 ml into the packed bed which is then allowed to sit for 1-2 minutes, etc.

SAMPLE WASHING

Wash bed with 1 ml of *Binding* Solution 3x in order to elute salts and other nonretained components.

SAMPLE RELEASE:

Wash bed with 1.2 ml of *Releasing* solution 2x (bed volume is 1.2 cc), allowing each portion to sit in the cartridge at least 1 minute before being pulled through with the vacuum. Repeat with a third 1.2-ml portion in order to elute all of the adsorbed solutes, combining the eluates (**NOTE:** It's a good idea to prewash collection tubes to eliminate leachable contaminants). Evaporate the solvent or proceed directly to the next analysis.

SELECTIVE ABSORPTION:

Binding is likely to be selective if solutes are present in great excess over the binding capacity. In that case, solutes that bind with high affinity will displace solutes that bind with lower affinity.