UptiTip™ Packed HILIC

Sample Preparation Extraction (SPE) of micro quantities.
Universal trapping of polar compounds

Catalog # : CH7060 1-10 µl
CH7070 10-200 µl

Name: **UptiTip™ Packed HILIC**

Applications : Universal trapping and concentration of peptides, some proteins, and other polar solutes, removal of salts and detergents. Works better with peptides, not with large proteins. Elution order is least to most polar (the opposite of reversed phase). The slit in the bottom, ~ 1-2 µm wide, permits fluids to pass but not the 12-µm packing material. Thus, no filter is necessary. This permits the elution of peptides in minimal volumes and minimizes the potential for sample loss or contamination. The capacity of this item is for samples 10-200 µl.

Directions for use

Recommended Solvents
Binding solution:
a) For peptides or polar small molecules : 15 mM ammonium acetate, pH 3.5,
with 85% acetonitrile (ACN).
b) For proteins with solubility problems : 50 mM formic acid with 75% propanol (PrOH) or PrOH:ACN = 1:1. If this does not suffice to maintain the proteins in solution, add 50 mM hexafluoro-2-propanol (HFIP) to this solution as well as to the sample.
Releasing solution : Same as binding solution but with 0-10% organic solvent (ACN or PrOH).

Conditioning Procedure
a) Tap the UptiTip gently to displace any packing material sticking to the top white cap. Remove the white caps from top and bottom.
b) Via a pipette tip inserted in the top of the UptiTip, add 5 µl (50 µl for # CH7070) of the releasing solution in order to wet the packing material. Attach the UptiTip to a pipettor or syringe and apply air pressure to force the solution through the packed bed. Remove the UptiTip from the pipettor and repeat this washing procedure 2-3x. Now wash the material 3x with the binding solution.

NOTE : Do not aspirate (suck up) the liquid. Since there is no filter on top, this will disrupt the packed bed and the material may be sucked into the pipettor. Liquids should always be forced through the packed bed either via positive air pressure or in a microcentrifuge.

Sample Binding
Apply the sample solution as above, attach the UptiTip to a pipettor or syringe, and force the liquid through the packed bed.
NOTE : If this process is slow, then hold the UptiTip onto the pipettor or syringe with one hand and push the plunger slowly; otherwise, the tip could pop off due to the high pressure. IMPORTANT : TO INSURE GOOD BINDING, SAMPLE SHOULD CONTAIN ABOUT THE SAME LEVEL OF ORGANIC SOLVENT AS THE BINDING SOLUTION.

Sample Washing
Wash the packed bed 2-3x with 10 µl (50 µl for # CH7070) volumes of the binding solution in order to elute salts, detergents, and other non retained components.

Sample Release
Wash the packed bed with 2-10 µl (25-50 µl for # CH7070) of releasing solution (bed volume is 8 µl (25 µl for # CH7070)). Repeat and combine the eluents in order to elute all of the adsorbed peptide. Evaporate the solvent or proceed directly to the next analysis.

Protocol of use

A sample can be loaded only from the top of the tip. Please don't try to pull the sample from the bottom of the tip. It works only in one direction.
SpinColumn-in-a-Tip : UptiTips can also be used as a Spin column-in-a-Tip by using the centrifuge adapters. Tips should be centrifuged at speeds between 2000-5000 rpm. The sample will be ready in a few minutes.

a) The UptiTip™ should be gently tapped so that the dry material does not stick to the white cap.
b) Remove the white cap from top and bottom of the UptiTip.
c) Place 50 µl of 70% Isopropanol or 70% Acetonitrile solution to wet the chromatographic material. Addition of 0.05% of TFA or other salt or ion-pairing salts can help in the binding of some protein or peptides for your specific application. **(Please do not pull the sample from the lower end of UptiTip™ as it will disturb the chromatographic media bed. Further more do not interrupt the pushing of the sample in between, as it will cause back pressure and the chromatography media may be sucked into the pipette and disturb the separation. Therefore once you start to press the sample, press all the way to the end until the entire sample has passed through the chromatographic bed. In case you have to interrupt the pressing, first remove the UptiTip™ from the pipette or syringe and then release the pressure of the pipette or syringe).**
d) Wash the chromatographic media bed volume 2-3 times with the solution of your choice.
e) Place the sample in the UptiTip and push with the micropipette. In case the solution passes very slowly through the UptiTip, use the syringe supplied with the UptiTips. In that case, hold the tip with one hand firmly and push the syringe slowly, otherwise the tip may pop out due to the pressure and you may lose your sample.
f) Wash the sample to remove salt or any undesired molecule with water or buffer or other solution for your specific applications.
g) Elute the sample with the elution buffer.

NOTE :

a) The UptiTip does not contain any filters therefore add sample very carefully so that the upper layer of chromatographic material bed is not disturbed.
b) Once you start pressing the pipette to enable sample to pass through the chromatographic bed, don't relieve the pressure in between. First remove the UptiTip from the pipette and then release the press button . If this is not done carefully, the chromatographic bed can be pulled into the pipette and will result in a loss of the sample or the chromatographic bed. This will also disturb the separation quality.
c) Small air bubbles in the bed do not disturb the separation.
d) At times, some peptides or proteins may be absorbed at the filter, however most of time they elute with the elution solution and do not stick to the filter.

CONCENTRATION OF SAMPLES :
The UptiTip is prepared as above. The dilute solution of biomolecules is passed with the help of the supplied syringe. The syringe supplied is 2.5 ml.

The sample should be pushed slowly and gradually and not more than a couple of hundred microliters per minute. A faster flow can also be achieved, if binding affinity of the biomolecule is high towards the chromatographic materials.

Once the biomolecule is bound to the chromatographic material, wash with water or buffer and elute the molecule in a very small volume. By this method, large volumes of several hundred ml samples can be concentrated to few micro liters.

For in vitro R&D use only

Please contact Uptima – Interchim for any other information

interchim
211 bis Av. J.F. - BP 1140 03103 Montluçon Cedex - Tel. 33 (0) 4 70 03 88 55 - Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com - web www.interchim.com