UptiTip™ Packed Silica IMAC

Sample Preparation Extraction (SPE) of micro quantities.
Recombinant protein purification.

Catalog # :	BI5460	1-10 µl	96 U
	BI5470	10-200 µl	96 U

Name : **UptiTip™ Packed Silica IMAC**

Applications : Affinity isolation of peptides and possibly proteins with phosphate or numerous histidine residues. The slit in the bottom, ~ 1-2 µm wide, permits fluids to pass but not the 50-µm packing material. Thus, no filter is necessary. This permits the elution of peptides in minimal volumes and minimizes the potential for sample loss . The estimated capacity of this item is ~ 50 µg (#BI5460), ~ 250 µg (#BI5470) protein or peptide, and sample volumes in the range 10-50 µl (#BI5460), 10-200 µl (#BI5470).

Directions for use

Buffers :

A. Wash Buffer-1 : 0.1% acetic acid in 60% acetonitrile
B. Wash Buffer-2 : 5% acetic acid in 5% acetonitrile
C. Water
D. Binding Solution : 50mM MES Buffer (pH5.5)
E. Releasing Solution : 0.4N Ammonium hydroxide
F. Metal ion loading Solution

1) Metal ions such as sulfate or chloride salts (for example Cupric Sulfate, Nickel Chloride, Gallium Nitrate) concentration 200mM in weakly acidic solutions (pH 5-6) because of solubility problems at higher pH.
2) Fe^{3+} should be loaded in more acidic conditions (pH 2-4) because of solubility problems at high pH.

Charging the tips with metal ions.

a) Tap the UptiTip™ Packed 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™ Packed [Fig. 2], add 10 µl of Wash buffer (A) in order to wet the IMAC material. Attach the UptiTip™ Packed to a pipettor or syringe and apply air pressure to force the solution through the packed bed. Remove the UptiTip™ Packed from the pipettor and repeat this washing procedure 3x.
c) Charge with the metal solution same as step (b). Remove the UptiTip™ Packed from the pipettor and repeat this washing procedure 3x.
d) Wash the Tip with 10ul of distilled water (C). Wash the Tip 3X with wash buffer (B). Equilibrate with binding buffer (D) 3X.

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. If this process is slow, then hold the UptiTip™ Packed onto the pipettor or syringe with one hand and push the plunger slowly ; otherwise, the tip could pop off from the pressure.

Protocol of use

Sample Loading (IMPORTANT : LOAD SAMPLE VERY SLOWLY (1-10ul/min.))
Load sample solutions as above. The eluate can be reloaded on the column if there is concern about the thoroughness of binding. Best results are generally obtained with loading solutions in the pH range 7-8, but 4-8.5 can be used. Acetate and phosphate buffers often result in strong binding. Buffers containing primary amines (such as Tris) often weaken binding and can strip metals.

Sample Washing
Wash the packed bed 2-3x with 10 µl volumes of the loading solvent in order to wash out nonretained proteins and peptides.

Sample Release
Wash the packed bed with 5-10 µl of releasing solution (E) (bed volume is 10 µl). Repeat 3x and combine the washes in order to elute all of the adsorbed protein or peptide

The sample can be desalted either through reversed-phase SPE or hydrophilic interaction chromatography SPE, depending on its polarity.

Selective Adsorption : **Binding is likely to be selective if peptides are present in great excess over the binding capacity. In that case, peptides or proteins that bind with high affinity will displace those that bind with lower affinity.**

For in vitro R&D use only

Please contact Uptima – Interchim for any other information

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