BioPureSPN™ Mini Graphite Glycan & Phosphopeptide Clean-up Columns & 96-Well Plates (5 µg glycan, 200 µg phosphopeptide approximate capacity)

Spin columns of Activated Graphite retain polar solutes such as released glycans and phosphopeptides. Salts, urea and non-polar solutes will not be retained permitting lower levels of detection by LC-MS or MALDI. Use of 0.1% TFA to acidify the resin will increase the binding of phosphopeptides. Ionic detergents must be removed to preserve capacity.

Directions: ***(p/n: HUM AC, HUM AC.20, HNS AC-U)****: Cut Off The Outlet Tab with Side-Cut Pliers or a Box Cutter against an elevated support surface. Loosen The Cap when spinning.*

Prior to use, the 20mg packing (~20µL void) should be activated using one tube volume (~400µL) alkali (e.g., 50% (v/v) ACN: 50% (v/v) water 0.1% (w/v) NaOH), then neutralized with one tube volume acid (e.g., 50% (v/v) ACN: 50% (v/v) water 1.0% (v/v) TFA). Wash with a one tube volume of 80% (v/v) acetonitrile water 0.1% (v/v) TFA followed by equilibration with one tube volume of 100% water.

Note: When using fixed-angle rotors, place a mark on the upper side of the column. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps. Improper orientation will result in reduced recovery efficiency. Also too high a spin speed will decrease the binding and/or elution effectiveness.

Desalting and purification of oligosaccharides and their derivatives

Clean-up of oligosaccharides, hydrazones or alditols released from glycoproteins solutions containing salts, detergents, proteins and release reagents (hydrazine and sodium borohydride). This procedure also can be used to fractionate neutral oligosaccharides from acidic (sialylated, sulfated or phosphorylated) oligosaccharides.

Aqueous solutions to be desalted are applied to the SPE Graphite columns and allowed to run into the adsorbent. The volume of the sample is not critical and the flow rate should be slow. Salts are washed off with approximately three chromatographic void volumes (CV) of water, while the glycans are adsorbed to the Graphite. Elute glycans with 60µL steps of 10% - 30% ACN in water or dilute acid.

Desalting and purification of phosphopeptides

Column Preparation

1. Remove top cap and bottom tab, place Graphite column into a 2mL collection tube and centrifuge at 2000 × *g* for 1 minute to remove residual water from the activation and cleaning steps (above).
2. Add 100µL of 1M NH_4OH and centrifuge at 2000 × *g* for 1 minute. Discard the flow-through. Repeat this step once.
3. Activate graphite by adding 100µL of acetonitrile. Centrifuge at 2000 × *g* for 1 minute and discard flow-through.
4. Add 100µL of 1% TFA and centrifuge at 2000 × *g* for 1 minute. Discard flow-through. Repeat this step once.

Sample Binding and Elution

1. Place Graphite column into a new collection tube and apply sample on top of the resin bed . Wait 2 minutes for the liquid to penetrate the bed.
2. Centrifuge at 1000 × *g* until all liquid is expelled. Discard the flow-through.
3. Wash the sample of salts and non-polar contaminants by adding 200µL of 1.0% TFA and centrifuging at 1000 × *g* for 1 minute. Discard the flow-through. Repeat this step once.
4. Place column into new collection tube. Add 100µL of 0.1% formic acid in 50% acetonitrile to elute sample. Centrifuge at 2000 × *g* for 1 minute. Repeat this step three more times using the same collection tube for a total elution volume of 400µL.
5. Gently dry sample in a vacuum evaporator. For MALDI-MS analysis, carefully suspend sample in 1-2µL of matrix solution prepared just before use. For LC-ESI applications, suspend sample in 0.1% FA or the appropriate buffer.

The Nest Group, Inc. TEL# 508-481-6223 FAX# 508-485-5736
17 Hayward St, Ipswich, MA 01938-2041 http://www.nestgrp.com E-mail: sales@nestgrp.biz

BioPureSPN™ Midi Graphite Glycan & Phosphopeptide Clean-up Columns & 96-Well Plates (12 µg glycan, 500 µg phosphopeptide approximate capacity)

Spin columns of Activated Graphite retain polar solutes such as released glycans and phosphopeptides. Salts, urea and non-polar solutes will not be retained permitting lower levels of detection by LC-MS or MALDI. Use of 0.1% TFA to acidify the resin will increase the binding of phosphopeptides. Ionic detergents must be removed to preserve capacity.

Directions: ***(p/n: HEM AC, HEM AC.20, HNS AC-M)****: Snap Off The Outlet Tab & Loosen The Cap*

Prior to use, the 50mg packing (~50µL void) should be activated using one tube volume (~400µL) alkali (e.g., 50% (v/v) ACN: 50% (v/v) water 0.1% (w/v) NaOH), then neutralized with one tube volume acid (e.g., 50% (v/v) ACN: 50% (v/v) water 1.0% (v/v) TFA). Wash with a one tube volume of 80% (v/v) acetonitrile water 0.1% (v/v) TFA followed by equilibration with one tube volume of 100% water.

Note: When using fixed-angle rotors, place a mark on the upper side of the column. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps. Improper orientation will result in reduced recovery efficiency. Also too high a spin speed will decrease the binding and/or elution effectiveness.

Desalting and purification of oligosaccharides and their derivatives

Clean-up of oligosaccharides, hydrazones or alditols released from glycoproteins solutions containing salts, detergents, proteins and release reagents (hydrazine and sodium borohydride). This procedure also can be used to fractionate neutral oligosaccharides from acidic (sialylated, sulfated or phosphorylated) oligosaccharides.

Aqueous solutions to be desalted are applied to the SPE Graphite columns and allowed to run into the adsorbent. The volume of the sample is not critical and the flow rate should be slow. Salts are washed off with approximately three chromatographic void volumes (CV) of water, while the glycans are adsorbed to the Graphite. Elute glycans with 150µL steps of 10% - 30% ACN in water or dilute acid.

Desalting and purification of phosphopeptides

Column Preparation

1. Remove top cap and bottom tab, place Graphite column into a 2mL collection tube and centrifuge at 2000 × *g* for 1 minute to remove residual water from the activation and cleaning steps (above).
2. Add 100µL of 1M NH4OH and centrifuge at 2000 × *g* for 1 minute. Discard the flow-through. Repeat this step once.
3. Activate graphite by adding 100µL of acetonitrile. Centrifuge at 2000 × *g* for 1 minute and discard flow-through.
4. Add 100µL of 1% TFA and centrifuge at 2000 × *g* for 1 minute. Discard flow-through. Repeat this step once.

Sample Binding and Elution

1. Place Graphite column into a new collection tube and apply sample on top of the resin bed . Wait 2 minutes for the liquid to penetrate the bed.
2. Centrifuge at 1000 × *g* until all liquid is expelled. Discard the flow-through.
3. Wash the sample of salts and non-polar contaminants by adding 150µL of 1.0% TFA and centrifuging at 1000 × *g* for 1 minute. Discard the flow-through. Repeat this step once.
4. Place column into new collection tube. Add 150µL of 0.1% formic acid in 50% acetonitrile to elute sample. Centrifuge at 2000 × *g* for 1 minute. Repeat this step three more times using the same collection tube for a total elution volume of 450µL.
5. Gently dry sample in a vacuum evaporator. For MALDI-MS analysis, carefully suspend sample in 1-2µL of matrix solution prepared just before use. For LC-ESI applications, suspend sample in 0.1% FA or the appropriate buffer.

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