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A Geno Technology, Inc. (USA) brand name

C18 Spin Columns

For Purification and Concentration of Peptide Samples

(Cat. # 786-930, 786-931)

INTRODUCTION

G-Biosciences C18 Spin Columns are ready-to-use micro centrifuge columns for peptide clean up and concentration. The columns consist of porous C18 reverse-phase resin that has a particle size if ~15µm and a pore size of 300Å. The resin offers highly efficient binding and recovery of peptides and is ideal for mass spectrometry and other peptide related applications.

Each spin column can be used to process between 10 to 150µl peptide samples in about 30 minutes without the need for specialized equipment. Each column can bind between 10ng to 30µg of protein peptides, although sensitivity and detection limits are dependent on selected downstream applications.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-930	C18 Spin Columns	25 columns
786-931	C18 Spin Columns	50 columns

STORAGE CONDITIONS

Shipped at ambient temperature. Upon receipt store at room temperature.

SPECIFICATIONS

- **Binding Capacity:** 10ng-30µg protein peptides
- **Volume Capacity:** 10-150µl
- **Support**:C18 coated silica gel
- **Particle size:** 15µm
- **Pore size:** 300Å

ADDITIONAL COMPONENTS REQUIRED

- Ultrapure water (G-Biosciences Proteomic Grade Water, Cat. # 786-229)
- Acetonitrile (ACN)
- Tirfluoroacetic acid (TFA)
- 1.5ml microcentrifuge tubes for collection tubes
- Methanol
- Bench top microcentrifuge (Up to 3,000xg)

IMPORTANT INFORMATION

- The lower level of detection for a protein is 20ng (300fmol). Each singular peptide, at this lower level of detection, needs to be at least 0.5ng to be detected effectively.
  NOTE: *Sensitivity and detection limits are dependent on selected downstream applications*
- Free, excess organic solvents (acetonitrile (ACN) or methanol) must be removed for optimal binding to G-Biosciences C18 spin columns. Simply air dry the sample in a vacuum evaporator and then carefully resuspend in 20µl 0.5% TFA in 5% ACN.
- Avoid excessive drying of the resin between steps.
- Plastics, including collection tubes and pipette tips, used in the procedure may introduce contaminants that interfere with mass spec analysis and other downstream applications. Use high quality plastics (Proteomic Grade Tubes, Cat. # 786-300). Alternatively, treating with Protein-OUT™ (Cat. # 786-680), a unique solution to remove proteins and other mass spectrometry interfering agents

PREPARATION BEFORE USE

- **Activation Solution**: 50% Methanol, 400µl/sample
- **Equilibration Solution**: 0.5% TFA in 5% ACN, 400µl/sample
- **Sample Buffer**: 2% TFA in 20% ACN, 1µl for every 3µl sample
- **Wash Solution**: 0.5% TFA in 5% ACN, 400-800µl/sample.
  NOTE: *Wash volume is dependent upon amount and type of contaminants present in the sample*
- **Elution Buffer**: 70% ACN, 40µl/sample
  NOTE: *Acceptable elution buffers include 50-70% ACN with or without 0.1% TFA, 50-70% methanol with or without 0.1% TFA. 0.1% formic acid can replace the TFA in ESI-MS applications.*

PROTOCOL

A. Sample Preparation

1. Mix 3 parts sample with 1 part Sample Buffer to give a final concentration of 0.5% TFA in 5% ACN.
   NOTE: *Each column can only process 10-150µl diluted sample.*

B. Prepare C18 Spin Columns

1. Tap the column to ensure the resin is settled in the base of the column, and then remove the top and bottom caps. Place column in clean collection tube.
2. Add 200µl Activation Solution (50% Methanol) to rinse the walls of the column and wet the resin.
3. Briefly centrifuge (1,500xg for 1 min) and discard the Activation Solution. Repeat the Activation Solution wash once.
4. Add 200µl Equilibration Solution (0.5% TFA in 5% ACN), centrifuge as before and discard solution. Repeat Equilibration Solution wash once.

C. Peptide Binding

1. Transfer the column to a clean collection tube and apply the sample from section A1 to the top of the resin bed.
2. Centrifuge at 1,500xg for 1 minute and recover the flow-through.
3. Reapply the flow-through to the resin bed to ensure complete binding. Repeat the centrifugation.
   NOTE: *Retain the final flow-through to confirm sample binding.*

D. Wash & Peptide Recovery

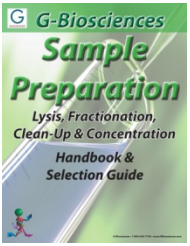
1. Transfer the column to a clean collection tube and apply 200µl Wash Solution (0.5% TFA in 5% ACN) to the resin.
2. Centrifuge at 1,500xg for 1 minutes and discard the flow-through. Repeat the wash step once.
   NOTE: *If sample contains high levels of contaminants then repeat wash step 1-2 times more. Contaminants, such as 2M urea and >100mM ammonium bicarbonate, will require these additional washes.*
3. Transfer the column to a clean collection tube. See Important Information section about plastics.
4. Add 20µl Elution Buffer (70% ACN) to the resin bed and centrifuge at 1,500xg for 1 minute. Repeat the elution step once.
5. Dry the sample in a vacuum evaporator and then proceed with your established methods.

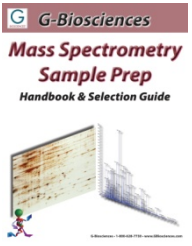
TROUBLESHOOTING

Issue	Possible Cause	Solution
Incomplete or poor peptide binding	High pH; lack of ion-pairing agents	Ensure TFA was added (Section A1)
	Excess organic solvent was present	Dry sample and resuspend in 20µl 0.1-0.5% TFA
	Sample lacks hydrophobicity to bind C18 resin	None
	Resin dried out before sample addition	Ensure resin does not dry out during the procedure. If necessary, keep resin in Equilibration Solution until sample ready to add.
Incomplete or poor peptide recovery	Highly hydrophobic sample	Use 70% ACN with 0.1% TFA to elute
	Sample loss due to non-specific interactions	Binding to plastics can cause significant loss at very low peptide concentrations. Minimize contact with plastics and storing at<300fmol concentration.
	Detection limits of application	Ensure sample is within the detection limit of downstream application.

RELATED PRODUCTS

Download our Sample Preparation and Mass Spectrometry Sample Prep Handbooks.





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