# 反向微柱的准备 Preparation of Reversed-Phase Microcolumns

## INTRODUCTION

One versatile strategy for sample cleanup prior to MALDI-MS analysis uses microscale columns designed for direct sample elution onto the MALDI target plate. This protocol describes the fabrication of a reversed-phase microcolumn designed for this purpose. The microcolumns are prepared from GELoader tips. This protocol has been optimized for sample cleanup prior to MALDI-MS. However, with slight modifications, it works equally well with samples destined for ESI-MS.

## MATERIALS

### Reagents

IMPORTANT: All reagents used in this protocol must be sequence grade.

Acetonitrile

Chromatography resins (Poros R1, R2, or Oligo R3, Applied Biosystems)

Formic acid (1%) (Optional, see note to Step 4)

Protein matrix solution (optional; see note to Step 8)

SA (sinapinic acid)

DHB (2,5-dihydroxybenzoic acid)

TFA (trifluoroacetic acid)

Acetonitrile

Dissolve SA or DHB in 50% acetonitrile containing 0.1% TFA.

Peptide matrix solution (optional; see note to Step 8)

Acetonitrile

CHCA ({alpha}-cyano-4-hydroxy-cinnamic acid)

TFA (trifluoroacetic acid)

Dissolve CHCA in 70% acetonitrile containing 0.1% TFA.

Methanol

Protein or peptide sample to be analyzed by mass spectrometry

Trifluoroacetic acid (TFA) (0.1%) (optional; see note to Step 4)

Equipment

Forceps, blunt tip (optional; see Step 1)

GELoader pipette tips (Eppendorf)

MALDI-MS target

Pipette tip (disposable, 20-200 µL size)

Syringe (1 mL)

Tubes (microcentrifuge, 1.5 mL)

METHOD

Partially constrict a GELoader pipette tip by squeezing the narrow end. The two most common ways to do this are illustrated in Figure 1 and listed below:

Figure 1. Methodology for preparing and using GELoader tip microcolumns. (a) Preparation of a constricted GELoader tip. (b) Generation of the column, application of the analyte sample, and elution of the analyte molecules.

Method 1: Place the narrow end of a GELoader tip flat on a hard surface. Roll a 1.5-mL microcentrifuge tube over the final 1 mm of the tip.

Method 2: Squeeze the narrow end of a GELoader tip using blunt forceps. To close the end, turn the tip once while holding it with the forceps.

Prepare a slurry of 100-200 µL of chromatography resin in 70% acetonitrile (use ~1.5 mg of resin/100 µL of acetonitrile). Steps 3-8 are illustrated in Figure 1.

Load 20 µL of 70% acetonitrile in the top of the constricted GELoader tip, and add 0.5 µL of the resin slurry on top of the acetonitrile. Use a 1-mL syringe fitted to the GELoader tip with a disposable pipette tip to gently press the liquid down to create a small column at the end of the constricted microcolumn. Dry the column by letting all of the liquid escape from the bottom of the column before performing the next step.

The disposable pipette tip must be cut twice to fit both the syringe and the GELoader tip. The amount of resin-slurry used to create the column should be varied with the approximate concentration of the sample. In general, the column height should be 1-6 mm (approximate bed volume is 10-60 nL) when working with peptides generated from poorly abundant, gel-separated proteins.

Apply 20 µL of 0.1% TFA to the top of the column. Equilibrate the column by gently pushing 10 µL of 0.1% TFA through it, using gentle air pressure generated by the syringe. The remaining 10 µL of 0.1% TFA should remain on top of the column bed. When the microcolumn is used as a cleanup step prior to nano-ESI-MS, 1% formic acid should be used instead of TFA, which is incompatible with ESI-MS.

Apply the protein/peptide sample on top of the remaining 10 µL of 0.1% TFA.

Press the liquid gently through the column by applying air pressure with the syringe. Do not allow the column to dry out; leave ~2 µL of solution on top of the column bed.

Wash the column with 20 µL of 0.1% TFA, and allow the column to run dry.

Elute the analytes, using 0.5 µL of matrix solution, directly onto the MALDI-MS target. The 0.5 µL of matrix solution should be spotted as several droplets (5-10) on the target. Alternatively, if the analytes are to be analyzed by ESI-MS, elute the peptides from the column using methanol/formic acid/H2O (50:1:49) either directly into the capillary needle or into a microcentrifuge tube.

The preferred MALDI matrices used for eluting protein from the column are either SA or DHB dissolved in 50% acetonitrile/0.1% TFA (see recipe for protein matrix solution). For eluting peptides from the column, the peptide matrix solution is preferentially used. Alternatively, the analytes can be eluted directly into a microcentrifuge tube for storage or further analysis, using any percentage of organic solvent. When eluted in several small spots, only the first two or three spots will contain the analytes, resulting in a further concentration of the sample.

The column can be reused after washing it extensively with 100% acetonitrile. Depending on the size of the column and the abundance/concentration of the analyte molecules that have been loaded onto it, the column can be reused two to ten times without observing any memory effects.

## REFERENCES

Gobom, J., Nordhoff, E., Ekman, R., and Roepstorff, P. 1997. Rapid micro-scale proteolysis of proteins for MALDI-MS peptide mapping using immobilized trypsin. Int. J. Mass Spectrom. Ion Proc. 169/170: 153-163.

Kussmann, M., Lassing, U., Sturmer, C.A., Przybylski, M., and Roepstorff, P. 1997. Matrix-assisted laser desorption/ionization mass spectrometric peptide mapping of the neural cell adhesion protein neurolin purified by sodium dodecyl sulfate polyacrylamide gel electrophoresis or acidic precipitation. J. Mass Spectrom 32: 483-493.