

Purification of peptide mixtures with stage-tip

Protocol: Manufacture of C₁₈-StageTip

Reagents

- Methanol

Method

1. Place an Empore Disk C18 (product number 2215, 3M, Minnesota, MN) on a flat, clean surface such as a disposable plastic petri dish.
2. Wet the membrane using 50 μ L methanol and keep it wet through out the protocol.
3. Punch out a small disk using a blunt-tipped hypodermic needle. The disk sticks in the needle and can be transferred into a pipette tip.
4. Push the disk out of the needle and fix it in the tapering of a pipette tip by a piece of fused silica or tubing fitting in the inside of the needle.
5. StageTips can be stored dry at room temperature.

Protocol: Use of C₁₈-StageTips for peptide isolation

Reagents

- Methanol
- 2% trifluoroacetic acid (TFA)
- Buffer A (0.5% acetic acid)
- Buffer B (80% acetonitrile, 0.5% acetic acid)

Method

1. Acidify the digested peptide sample using 2 % TFA. For a 10 mM TrisHCl (pH 8.0) buffered solution 1/10 volume is sufficient. Check with pH strips.
2. Trim the stagetip to fit on a 1 mL plastic syringe.
3. Condition a C₁₈-StageTip by placing 50 μ L Buffer A from the top and pressing the liquid through at 50 μ L/min using a 1 mL plastic syringe. Repeat this subsequently with 50 μ l buffer B and 50 μ l methanol.
4. Equilibrate the StageTip using 50 μ L buffer A at 50 μ L/min. Repeat this once.
5. Load sample at 20 μ L/min.
6. Wash with 50 μ L buffer A at 50 μ L/min.
7. Elute using 2-5 μ L buffer B at 10 μ L/min and dilute ten times by adding buffer A.
8. Directly load onto a pre-column or an analytical column of an LC-MS system for analysis. Reduce the volume if required by dry down in a vacuum centrifuge; however, do not dry down completely.