

SDB-RP - StageTip purification of peptides

Reagents & Solutions

- Buffer A: 0.1% formic acid in water *
- Buffer B: 0.1% formic acid in 80% Acetonitrile *
- Formic acid, 10% in water *
- 100% Methanol *

Equipment

- SDB-RP StageTips, packed in 200 µL pipette tips *
- 2 mL Eppendorf collection tubes *
- Centrifugal adaptors for StageTips *
- Eppendorf benchtop centrifuge *

* available in the core facility

Protocol

StageTip conditioning & equilibration

1. Use one new StageTip per sample (containing 2 layers of SDB-RPS discs)
2. Equilibrate the StageTips¹:
 - add 20 µL Methanol to the StageTip → centrifugation at 2.600 rpm for 1 min
 - add 20 µL buffer B to the StageTip → centrifugation at 2.600 rpm for 1 min
 - add 20 µL buffer A to the StageTip → centrifugation at 2.600 rpm for 1.5 min
 - add 20 µL buffer A to the StageTip → centrifugation at 2.600 rpm for 2 min
 - make sure to keep a little bit (approximately 1-2 mm) of buffer A on top of the C18 material. If too much buffer A remains on top centrifuge ONLY THESE StageTips a little bit longer²
3. Discard the solvents from the collection tubes before you continue

Sample purification

4. Centrifuge the samples (acidified with formic acid) at full speed for 5 min
5. Load the samples onto the StageTips. Only use the supernatant in case a pellet is visible after centrifugation
6. Centrifugation at 2.600 rpm for 5 min²
7. Wash the StageTips with 30 μ L buffer **A** → centrifugation at 2.600 rpm for 3 min
8. Wash the StageTips with 30 μ L buffer **B** → centrifugation at 2.600 rpm for 3 min
9. Wash the StageTips with 30 μ L buffer **B** → centrifugation at 2.600 rpm for 3 min
10. Now dry the StageTip completely with a syringe & put the StageTips at 4°C

Notes

¹ Centrifugation times can vary, depending on sample volume, C18 phase tightness and sample composition. Adjust if necessary.

² IMPORTANT: The Stage tips should not be completely dry after this step! See also example pictures:

