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FASP-IP

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Protocol status: Working
We use this protocol and it's working

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Abstract

The Filter-Aided Sample Preparation (FASP) protocol enables efficient protein digestion and cleanup using membrane filters, eliminating detergents and contaminants prior to mass spectrometry.

Guidelines

Ensure all liquid has passed through the filter before proceeding to the next step.The membrane should remain visibly moist — it must have a wet sheen but never be completely dry. Drying can cause protein loss, membrane damage, or reduced recovery during subsequent washes and digestion steps.

Materials

Solutions:

DTT solution: 25 mM DTT in 50 mM ABC.
Precipitation buffer: 8M urea in 50 mM ABC
IAA solution: 55 mM IAA in 8M urea in 50 mM ABC.
ABC solution: 50 mM ABC

Materials:

FASP tubes: VN01H22 Sartorius

Safety warnings

ABC must be made fresh the same day of the experiment.
Urea will precipitate if exposed to high temperatures and prevent carbamylation

Reduction/Alkylation

1h 5m

1 Add DTT solution to the sample for a final concentration of 25 mM. Incubate at 80ºC for 15 min. 15m

2 Wait for the sample to cool down. Add urea to the sample to have a final concentration of 8M.
This can be either directly adding the urea powder to the solution, or diluting 5x with 8M urea in 50 mM ABC.

3 Spin sample through a 30k spin column until all the liquid goes through the filter, 15000r cf for 15. 15m

4 Add 100 µL of 55 mM IAA to the column. Incubate at Room temperature for 20 min in the dark. 20m

5 Spin through column at 15000 rcf for 15min. 15m

Wash

45m

6 Wash with 100 µL of 8 M urea in ABC. Spin through column at 15000 rcf for 15-20 min. 15m

7 Wash twice with 100 µL of ABC. Spin through column at 15000 rcf for 15min. 30m

Digestion

8 Make trypsin solution by adding 20 µL of 0.1 % TFA to a trypsin vial. Add 980 µL of ABC.

9 Place column into a new tube and add 100 µL of freshly prepared trypsin solution. Wrap the top of the tubes wit parafilm and incubate at 37 ºC O/N. Use a wet paper towel underneath the Eppendorf rack to minimise evaporation.