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In Gel Digestion

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We use this protocol and it's working

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Abstract

In-gel digestion is an effective alternative for processing protein samples containing contaminants such as detergents or other interfering reagents. However, it typically requires higher amounts of starting protein to achieve optimal results.

Guidelines

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Materials

Buffers
Ammonium Bicarbonate (ABC): Sigma Aldrich A6141-500G
Acetonitrile (ACN): ThermoFisher A955-122 (LC-MS Optima Grade)
Trifluoroacetic acid: TFA: Sigma Aldrich, T6508-25ML

Enzymes
Trypsin: ThermoFisher 90057 20 µg per vial

Reagents
DTT 1M Dithiothreitol (DTT): Sigma Aldrich, 43815-5G
IAA 1M Iodoacetamide (IAA): Sigma Aldrich: I1149-25G

Solutions
10 mM DTT in 50 mM ABC
55 mM IAA in 50 mM ABC

Troubleshooting

Problem

Incomplete digestion

Solution

Make sure the gel pieces have absorbed the trypsin solution before leaving them to incubate O/N. Add extra solution so there is an excess over the gel pieces

Safety warnings

Perform IAA incubation in the dark

Steps

30m

1 Cut excised bands into cubes 1 mm (carefully, clean scalpel between samples with ethanol). 30m

2 Carefully transfer gel pieces into a (1.5mL) microcentrifuge tube

Washing

1h

3 Add 100 µL ABC and 120 µL ACN and incubate @ 37 °C for 30min in a shaker set to 1000 rpm. 30m
Make sure the gel pieces are completely covered. If not, add additional ABC and ACN **in the same ratio** until fully covered.

4 Refresh buffers and repeat, the gel pieces should be faintly blue after the second wash. Repeat if the gel pieces are still too blue. 30m

Reduction/Alkylation

50m

5 Remove buffer mix from the washing step and cover the gel pieces with ACN. Remove ACN after 5 min. The gel pieces should be small and white before proceeding with the next step. Repeat this step if after 5 min the gel pieces have not shrunk. 5m

6 Cover gel pieces with 10mM DTT (in 50 mM ABC) solution (enough to cover the gel pieces). Incubate for 30 min at 37 °C in shaker set at 1000 rpm. Make sure the Eppendorf tubes are properly closed. 5m

7 Remove the DTT solution and add ACN to shrink the gel pieces (5min). Remove liquid. 5m

8 Add 55 mM iodoacetamide (IAA) (in 50 mM ABC) solution to the tube (enough to cover the gel pieces). 20m
Incubate for 20 min at room temperature in the dark. Remove the iodoacetamide solution.

9 Add 50 mM ABC buffer (enough to cover the gel pieces) and incubate at Room temperature for 5 min. Remove solution and discard in waste. 5m

10 Add ACN (enough to cover the gel pieces) until the pieces become white and shrink (~5 min). Remove solution and discard in waste. 5m

11 Add ACN (enough to cover the gel pieces) for 5min to **shrink** the gel pieces. Leave in until just before adding trypsin. 5m

Trypsin digestion 15m

12 Resuspend the lyophilised trypsin in 20 µL of 0.1 % TFA, add 1030 µL of dH2O, 300 µL of 50mM ABC and 150 µL of ACN 5m

13 Add trypsin (enough to cover the gel pieces) and leave it in the fridge for 5-15 min (5 min is enough). 5m

14 Incubate the sample @ 37 °C After 10 min, check if all liquid was absorbed and if necessary, add more trypsin. Gel pieces should be completely covered with trypsin buffer. 10m

15 Incubate sample overnight (12-16 hours) at 37 °C .