PSR In-gel Digestion

05/07/07

Reagents

diH_2O
50% 100 mM Ammonium Bicarbonate (AB) / 50% Acetonitrile (ACN)
100% 100 mM AB
100% ACN
10mM Dithiothreitol (DTT) in 100 mM AB
55mM Iodoacetamide (IAA) in 100 mM AB
100% 25 mM AB
Trypsin Digestion Solution (make fresh): Thaw a 50 µL vial of 0.1 ug/ µL trypsin, and add 200 µL of 100 mM AB, 4 µl 0.5 M $CaCl_2$, and 146 µL diH_2O
Digestion Buffer: 5.0 mL of 100 mM AB, 100 µL of 0.5 M $CaCl_2$, 4.90 mL H_2O

Pre-Digestion

Solutions removed after each incubation unless otherwise noted

(2x) Add diH_2O wash of whole gel for 15 min

Cut out gel bands and put into vials

(2x) Add 100ul of 50% 100 mM AB / 50% ACN and incubate for 30 min on shaker

Put sample in Speed-Vac to remove excess solution

Add 100 µL of 10 mM DTT and incubate for 45 min @ 56ºC

Add 100 µL of 55 mM IAA cover with foil and incubate for 30 min on shaker

Add 500 µL of 50% 100 mM AB / 50% ACN and incubate for 15 min on shaker

Put sample in Speed-Vac to remove excess solution

Digestion

Add 20 µL of Trypsin Digestion solution and incubate for 15 min @ 4ºC
If gel plug isn't completely re-swelled add an additional 10 µL of Trypsin solution and repeat the incubation until the gel plug won't absorb any more solution then remove any excess solution

Add 60 µL of Digestion Buffer and incubate @ 37ºC overnight, more digestion buffer can be added to cover the gel slices, don't remove solution after incubation

Short Extraction Method

Add 3 µL of neat FA added to the above solution, bath-sonicate for 15min at 37°C, pull off and save solution for mass spec analysis

Long Extraction Method

Pull off solution from the overnight digestion into a separate vial

Add 50 µL of 25 mM AB gel pieces, bath-sonicate for 15 min @ 37ºC, don't remove solution after incubation

Add 50 µL of 50% ACN to the above solution, bath-sonicate for 15 min @ 37ºC, remove supernatant after incubation and combine it with the solution from the overnight digestion

Concentrate the extracts to approx. 10 µL in a Speed-Vac (don't let them go dry) and save for mass spec analysis