

## PSR In-gel Digestion

03/28/11

### Reagents

diH<sub>2</sub>O

50% 100 mM Ammonium Bicarbonate (AB) / 50% Acetonitrile (ACN)

100% 100 mM AB (0.791 g into 100ml water)

100% ACN

10mM Dithiothreitol (DTT) in 100 mM AB (15.4 mg into 10 ml 100 mM AB)

55mM Iodoacetamide (IAA) in 100 mM AB (10 mg into 1.0 ml 100 mM AB)

100% 25 mM AB

Trypsin Digestion Solution (make fresh): Dilute 20ul of 0.1ug/ul trypsin (in trypsin buffer) with 180ul of 100mM AB

\*Volumes should be varied based on the amount of gel material present\*

\*Solutions removed after each incubation unless otherwise noted\*

### Pre-Digestion

(3x) diH<sub>2</sub>O wash of whole gel for 10 min

Cut out gel bands and put into vials. Slice into < 3 mm pieces if needed

(2x) Add 500ul of 50% 100 mM AB / 50% ACN and incubate for 30 min on shaker

Add 500ul of 100% ACN and incubate for 5 min on shaker

Put sample in Speed-Vac to dehydrate

Add 100 µL of 10 mM DTT and incubate for 45min @ 56°C (it may not be possible to remove solution after this step)

Add 100 µL of 55 mM IAA cover with foil and incubate for 30 min on shaker (it may not be possible to remove solution after this step)

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

Add 500 µL of 100% ACN and incubate for 5 min on shaker

Put sample in Speed-Vac to dehydrate

### Digestion

Add ~100 µL of Trypsin Digestion solution and let sit for 15 min on desktop (enough to re-swell gel)

Add 50 - 100  $\mu$ l of 100 mM AB (enough to cover gel slices) and incubate @ 37°C overnight, don't remove solution after incubation.

### Extraction

Pull off solution from the overnight digestion into a separate vial

Add 50-100  $\mu$ L of 25 mM AB to gel pieces, incubate in shaker for 15 min @ 37°C, don't remove solution after incubation

Add 50-100  $\mu$ L of 50% ACN to the above solution, incubate in shaker for 15 min @ 37°C, remove supernatant after incubation and combine it with the solution from the overnight digestion

Add 50-100  $\mu$ L of 5% Formic acid, incubate in shaker for 15 min @ 37°C, don't remove solution after incubation

Add 50-100  $\mu$ L of 50% ACN to the above solution, incubate in shaker for 15 min @ 37°C, remove supernatant after incubation and combine it with the solution from the overnight digestion

Take the collection of supernatants and filter through a 0.45 micron spin filter. Spin at 4000rpm for 5 min.

Take the samples to dryness in a Speed-Vac and save for mass spec analysis.