

In-Gel Digestion

- 1.) Excise band and cut into small pieces $\sim 1\text{mm}^2$, place in Eppendorf tube.
- 2.) Wash gel pieces for 15 min (gentle vortex) with 100 μL 100mM Ambic*/50% ACN, spin down, pull off supernatant and discard. Repeat if necessary until dye is removed.
- 3.) Pull off final wash solution, add 100 μL of 100% ACN, incubate at room temp for 5 min. Carefully remove ACN and air dry at room temp for 5 min. Since you must leave the cap of the tube open to air dry, cover them with something (e.g. pipette tip box top) to prevent dust from entering the tubes.
- 4.) Add 100 μL of DTT solution (10mM in 100 mM Ambic), incubate at $\sim 60^\circ\text{C}$ for 30 min.
- 5.) Spin down, remove supernatant, add 100 μL of IAA (55mM in 100 mM Ambic), incubate in the dark at room temperature for 30 min.
- 6.) Spin down, remove supernatant and wash/dehydrate for 5 min with 100 μL 100mM Ambic /50% ACN.
- 7.) Spin down, remove wash, add 100 μL of 100% ACN, incubate at room temp for 5 min. Carefully remove ACN and air dry at room temp for 5 min. Again, cover tubes to prevent dust contamination.
- 8.) Add 6 μL trypsin solution and 34 μL 100 mM Ambic (final trypsin concentration = 15 ng/ μL). Check samples after 20 min and add additional 100 mM Ambic if gel pieces absorb all the liquid.
- 9.) Incubate overnight at 37°C
- 10.) Spin down, pull off supernatant and save in a clean Eppendorf tube.
- 11.) Add 40 μL of extraction solution (50% ACN, 0.1% TFA) incubate 20 min with gentle vortex.
- 12.) Spin down, pull off supernatant, add to sample from step 12.
- 13.) Repeat extraction step.
- 14.) Speed Vac to almost dry, bring up in 10 μL 0.1% TFA.
- 15.) For MALDI-TOF/TOF analysis sample needs to be zip-tipped.
- 16.) For LC-MS/MS analysis bring sample up in 13 μL of 0.1% acetic acid, 5% ACN.

*Ambic = Ammonium Bicarbonate, NH_4HCO_3

Stock Solutions:

<u>1M Ambic</u>	<u>Trypsin (in -80 freezer)</u>
0.079g Ambic	0.1 $\mu\text{g}/\mu\text{L}$
1 mL ddH ₂ O	
(pH ~ 7.8)	

Solutions to prepare:

<u>100 mM AmBic/50% ACN</u>	<u>10mM DTT</u>	<u>100 mM AmBic</u>	<u>55mM IAA</u>
100 μL 1M AmBic	1.5 mg DTT	100 μL 1M Ambic	10 mg IAA
500 μL ACN	900 μL ddH ₂ O	900 μL ddH ₂ O	900 μL ddH ₂ O
400 μL ddH ₂ O	100 μL 1M Ambic		100 μL 1M Ambic