

Quick (relatively) Method for Preparing Gel Fragments for Mass Spec

Materials:

Acetonitrile (100% HPLC Grade) (ACN)

1 M Ammonium BiCarbonate (10X ABC)

100 mM ABC (pH 8-8.5 if made fresh)

2:1 100 mM ABC : ACN

10 mM DTT solution (10 uL of 1M DTT + 990 uL 100 mM ABC)

55 mM Iodoacetamide solution (100 uL of 550 mM Iodoacetamide + 900 uL of 100 mM ABC)

Trypsin (1 vial of Trypsin in 100 mM ABC of appropriate volume per sample)

- 1.) Cut up gel slice into pieces < 1 mm on a side and put in 2 mL tube
- 2.) Add 2:1 ABC : ACN and incubate 20 min at 25C and 750 rpm
- 3.) Discard super
- 4.) Repeat steps 2 & 3 (remove coomassie blue stain)
- 5.) Add ACN and incubate 5 min at 25C and 750 rpm
- 6.) Discard super
- 7.) Add 10 mM DTT Solution and incubate 1 hour at 30C and 750 rpm
- 8.) Discard super
- 9.) Add ACN and incubate 5 min at 25C and 750 rpm
- 10.) Discard super
- 11.) Add Iodoacetamide Solution and incubate in the dark (drawer) for 30 min
- 12.) Discard super
- 13.) Add ACN and incubate 5 min at 25C and 750 rpm
- 14.) Discard super
- 15.) Add 2:1 ABC : ACN and incubate 30 min at 25C and 750 rpm
- 16.) Discard super
- 17.) Repeat steps 14 and 15 (wash: remove last traces of coomassie blue stain)
- 18.) Add ACN and incubate 5 min at 25C and 750 rpm
- 19.) Discard super
- 20.) Add Trypsin and incubate 15 min on ice
- 21.) Put samples in 37C overnight
- 22.) Remove super from Overnight digestion and put in new tube
- 23.) Add 2:1 ABC : ACN and incubate for 20 min at 25C and 750 rpm
- 24.) Remove super and pool with digestion super
- 25.) Add ACN and incubate 5 min at 25C and 750 rpm
- 26.) Remove super and pool with digestion super
- 27.) Place samples in speed vac and run until dry
- 28.) Bring up in 100 uL of sample buffer each and transfer to Orbi-Trap or LTQ sample bottles.
- 29.) Setup run on machine and load samples.
- 30.) Check results and submit searches