

In-Gel Trypsin Digestion Protocol

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Suppliers and Catalog Numbers:

Ammonium Bicarbonate (EMD catalog #: 00113164 extra pure grade)
HPLC Water (Burdick&Jackson catalog #: 365-4)
HPLC Acetonitrile (Burdick&Jackson catalog #: 015-4)
DTT (Calbiochem catalog #: 233153 ultrol grade)
IAA (Sigma catalog#: A3221)
Acetic Acid (JTBaker catalog #: 9515-03)
TFA (Pierce catalog #: 28901 HPLC grade)
Trypsin (Promega catalog #: V5280 Trypsin Gold Mass Spectrometry Grade)
Protein Lobind Tubes (Eppendorf 1.5mL catalog #: 22 43 108-1)
(Eppendorf 0.5mL catalog #: 022431064)

Reagents:

100mM Ammonium Bicarbonate
395.3mg Ammonium Bicarbonate
Up to 50mL HPLC water
Store at Room Temperature

100mM Ammonium Bicarbonate in 50% Acetonitrile
395.3mg Ammonium Bicarbonate
25mL HPLC Acetonitrile
Up to 50mL HPLC Water
Store at Room Temperature

10mM DTT
7.71mg dithiothreitol (DTT)
Up to 5mL 100mM Ammonium Bicarbonate
MAKE FRESH

50mM IAA
Vial from Sigma contains 56mg iodoacetamide
Reconstitute in 6.06mL Ammonium Bicarbonate
MAKE FRESH

20mM Ammonium Bicarbonate in 50% Acetonitrile
10mL 100mM Ammonium Bicarbonate
15mL HPLC water
25mL HPLC Acetonitrile
Store at Room Temperature

40mM Ammonium Bicarbonate in 10% Acetonitrile
20mL 100mM Ammonium Bicarbonate
5mL HPLC Acetonitrile
25mL HPLC water
Store at Room Temperature

50mM Acetic Acid
144µL Acetic Acid

Up to 50mL HPLC water
Store at Room Temperature

Trypsin Solution
100µL 50mM Acetic Acid
Up to 5mL 40mM Ammonium Bicarbonate in 10% Acetonitrile
Aliquot into 500 µL Eppendorf Protein Lobind Tubes and store at -80C
Each Aliquot can undergo 5 freeze-thaw cycles

50% Acetonitrile/5% TFA
25mL HPLC Acetonitrile
2.5mL TFA
Up to 50mL HPLC Water

Procedure (Optimized for Coomassie and SYPRO stains):

1. Excise band from gel (cutting blade and forceps should be rinsed with 70% ethanol before and after each band).
2. Cut each band into 1 x 1 mm pieces and place in 1.5mL Lobind tube.
3. Rinse gel pieces with 300µL HPLC water for 15 min at room temperature on shaker.
4. Add 300µL HPLC Acetonitrile and wash for 15 min at room temperature on shaker.
5. Discard supernatant.
6. Wash gel pieces with 300µL 100mM Ammonium Bicarbonate for 15 min at room temperature on shaker.
7. Discard supernatant.
8. Add 300µL 100mM Ammonium Bicarbonate in 50% Acetonitrile for 15 min at room temperature on shaker.
9. Discard supernatant.
10. Add 100µL HPLC Acetonitrile for 5 min at room temperature on shaker.
11. Discard supernatant.
12. Dry gel pieces in a Speedvac for 5 min.
13. Add 50µL 10mM DTT and incubate for 1 hour at 60C
14. Discard Supernatant.
15. Add 50µL 50mM IAA and incubate for 30 min in dark at room temperature.
16. Discard supernatant.
17. Wash gel pieces with 300µL 100mM Ammonium Bicarbonate for 15 min at room temperature on shaker.
18. Discard supernatant.
19. Wash gel pieces with 300µL 20mM Ammonium Bicarbonate in 50% Acetonitrile for 15 min at room temperature on shaker.
20. Discard supernatant.
21. Add 100µL HPLC Acetonitrile for 5 min at room temperature on shaker.
22. Discard supernatant.
23. Dry gel pieces in a Speedvac for 5 min.
24. Add 20µL of Trypsin solution and incubate for 1 hour at room temperature.

25. Add enough 40mM Ammonium Bicarbonate in 10% Acetonitrile to completely cover the gel pieces.
26. Incubate overnight at 37C.
27. Add 150 μ L HPLC water to pieces for 10 minutes at room temperature on shaker.
28. Remove supernatant and place in a 0.5mL Lobind tube.
29. Extract the gel pieces twice with 50 μ L of 50% Acetonitrile/5% TFA for 60 minutes each time at room temperature.
30. Pool all extracts and dry in a Speedvac.
31. Purify and concentrate peptides using ZipTips (see "ZipTip Protocol").