2D Electrophoresis
Trypsin in-gel Digest (PSC 537)

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General Information There are numerous protocols for in-gel protein digestion - they are all variations on the same theme. This is the protocol that Kyu developed. The TFA has a limited shelf life. There is conflicting information about how long to use TFA once opened. Replace the TFA if it turns yellow or after 4 - 6 months. The Supplies section has order information about a 0.5 ml ampule of TFA, so you don't have to toss too much. One of the biggest problems with protein yield after in-gel digestion is when the peptides stick to the plastic - tips, tubes or plates. Included in the Buffers and Supplies section are some low-bind products that might help. Also, be careful not to dry the peptides completely. This can't be avoided with a microplate, though. So, again, think about using a low-bind plate.

Protein digestion 5/1/07

Notes: • Perform reagent prep and protocol in hood using nitrile gloves • Thoroughly rinse all reagent tubes with ddH2O

1. Process immediately after gel-plugs are picked.
2. Prepare first 3 reagent. Keep reconstituted trypsin at –20oC.
3. If plugs were collected using an Ettan Spotpicker, remove ddH2O - be careful not to smash the plugs. If your gel was destained with 40% MeOH/10% Acetic acid, proceed to step 7.
4. Add 400µl 50% MeOH/10% acetic acid. Incubate 60 min and remove solution.
5. Repeat step 4.
6. Optional for storage: add 10 – 15 µl 50% MeOH/10% Acetic acid to store the gel-plug; store o/n or days at 4oC.
7. Remove all liquid and add 400µl 50% ACN/100 mM NH_4CO_3. Incubate 1 hour with agitaion. Remove liquid.
8. Add 50µl 100% ACN. Incubate for 20 min. Remove liquid.
9. Dry plugs in speed vac (may need to remove rotor) for 1 h with no heat. Dried gel-plugs can be stored for days at –80oC)

10. When ready, make 1 X trypsin (20 ng/µl) from 10 X stock with 25 mM NH4CO3 (pH 8.0). Add small amount of 1 X trypsin solution to gel plug. Use 1 µl to 7 µl , depending on amount of protein in the gel-plug.

    a. The amount of trypsin is usually 60 - 120 ng/gel plug depending on the amount of protein in the gel.

    b. Keep the trypsin amount sub-molar ratio to protein.

    c. Bring final volume to 30 - 40 µl with 25 mM NH4CO3 (pH 8.0)

11. Seal plate and incubate at 37oC incubator overnight (14 – 16 hours).

12. Make up last two reagents.

13. Remove solution and put in new fresh plate/tube.

14. Add 30 - 40 µl 50% acetonitrile/0.2% TFA. Incubate 30min and transfer to the same plate/tube as above.

15. Add 30 - 40µl 50% acetonitrile/0.1% TFA. Incubate 30min and transfer to the same plate/tube as above.

16. Dry solution in speedvac (no heat) until almost dry, around 1h or less. Check periodically to prevent drying to completion. If using tubes, try to NOT dry to completion. Peptide fragments tend to stick to plastic (use low bind tubes). Dry until about 10 µl are left.

17. If you are going to clean up your sample with Zip-Tips, dry to 3 -5 µl and resuspend to 10 µl with 0.1% TFA.

18. Proceed to ziptip or freeze samples at -20C.

Buffers and Solutions

50% MeOH/ 10% acetic acid – 8ml
3.2ml ddH2O
4ml MeOH
0.8ml acetic acid

100 mM ammonia bicarb / 50% Acetonitrile – 8ml
3.84ml ddH2O
4ml ACN
800 µl 1 M ammonium bicarb (pH 8.0)

200ug/ml trypsin/Promega reconstitution buffer = 10 X stock
to 100 µl reconstitution buffer (200ng/ µl)
mix and aliquot 5µl/tube

25 mM ammonia bicarb - 5 ml
125 µl 1M ammonium bicarb (pH 8.0)
4.875 ml ddH2O

50% acetonitrile / 0.2% TFA – 4ml
2ml ddH2O
2ml acetonitrile
8ul 100% TFA

50% acetonitrile / 0.1% TFA – 4ml
2ml ddH2O
2ml acetonitrile
4ul 100% TFA

Supplies
1. Trypsin
Quantity/Unit: 100 ug/vial (5 X 20ug)
Vendor: Promega
Catalog#: V511A

2. TFA (Trifluoroacetic acid
Quantity/Unit: 1 Pkg (5 X 0.5 ml)
Vendor: Aldrich
Catalog#: 308897-1PAK

3. Siliconized microfuge tubes, 0.5 ml ($50.35/PK)
Quantity/Unit: 500/PK
Vendor: Applied Biosystems
Catalog#: AM12350

4. Siliconized microfuge tubes, 1.5 ml ($40/PK)
Quantity/Unit: 250/PK
Vendor: Applied Biosystems
Catalog#: AM12450

5. Low Retention microfuge tubes, 0.6 ml ($25/PK)
Quantity/Unit: 1000 (10 bags of 100)
Vendor: CLP, Continental Lab Products
Catalog#: 3435.S3

6. Low Retention microfuge tubes, 1.5 ml ($14/PK)
Quantity/Unit: 500

Vendor: CLP, Continental Lab Products
Catalog#: 3445.S3

7. Low Retention pipet tips, Racked and Pre-sterilized, 200 ul($25.50/PK)
Quantity/Unit: 10 trays of 96
Vendor: CLP, Continental Lab Products
Catalog#: 2102.YS

8. Low Retention pipet tips, Racked and Pre-sterilized, 10 ul($25.50/PK)
Quantity/Unit: 10 trays of 96
Vendor: CLP, Continental Lab Products
Catalog#: 2142.S

9. Low Bind microfuge plates, conical, Pre-sterilized
Quantity/Unit: 100
Vendor: PGC
Catalog#: 81-6664-56